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Comparative Evaluation of the Efficiency of *Hibiscus sabdariffa* on Obesity Management in Albino Rats in Sudan

Islam Adam Ahmed Daffalla[1]*, Shamseldein Hasabella Ahmed Abdalla[2], Selma Elmalieh Abdalla Salih[3]

[1]Ministry of Animals Resources and Fisheries, South Darfur State, Sudan

[2]Department of Biomedical Sciences, College of Veterinary Medicine, Sudan University of Sciences and Technology, Khartoum, Sudan

[3]Department of Biomedical Sciences, College of Veterinary Medicine, Sudan University of Sciences and Technology, Khartoum, Sudan

***Address for Correspondence:** Dr. Islam Adam Ahmed Daffalla, Ministry of Animals Resources and Fisheries, South Darfur State, Sudan
E-mail: islamahmedadam333@gmail.com

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ABSTRACT

Background: Researchers studying *Hibiscus sabdariffa* L. family Malvaceae calyx ethanol extract to determine its physiological activity-structure relationship are typically found in regions where it is utilised in traditional medical and food applications. This paper was to the evaluation of the Efficiency of *Hibiscus sabdariffa* extract (HSE) in treating obese rats.

Methods: Twenty-eight obese male albino rats were classified into 4 groups(seven of each), group (A) was healthy control rats were fed on a normal diet (ND), and Obese rats in B, C and D groups were fed with high-fat diet, group (C) rats were treated with 12 mg/Kg BW orlistat, group (D) obese rats were treated with 200 mg/Kg BW (HSE) Blood was collected from all groups at zero time and after 2,4 and 6 weeks of the experimental period.

Results: Treatment with a high-fat diet increased the body weight (g) and biochemical measurements of total cholesterol (TC), low-density lipoprotein (LDL), Alkaline Phosphatase (ALP), triglyceride, Alanine Aminotransferase (ALT), Aspartate Aminotransferase **(**AST) and very low-density lipoprotein (VLDL). Significant reductions in body weight were obtained by administration of *H. sabdariffa* extract in obese albino rats. Also, the levels of triglyceride, cholesterol, ALT, AST, ALP, VLDL and LDL were reduced in the treated group.

Conclusion: In this paper, obese rats treated with *H. sabdariffa* extract obtained a significant decrease in body weight, serum cholesterol, ALT, AST, TG and LDL when compared with the control 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.

Key-words: Albino rats, Body weight, High Fat diet, *Hibiscus sabdariffa*, Orlistate

INTRODUCTION

Obesity is a chronic metabolic disorder caused by an imbalance between energy intake and expenditure. It has become the sixth common cause of disease burden worldwide [1]. It is defined as an abnormal or excessive accumulation of fat that may put a person at health risk [2,3] to such an extent that may reduce life expectancy [4].

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Chronic obesity is a problem of epidemic proportions, which has an important impact on lifestyle-related diseases such as coronary heart disease, dyslipidemia, glucose intolerance, diabetes, hypertension and some types of cancers. Several factors, including lack of exercise, sedentary lifestyles and consumption of energy-rich diets contribute to the etiology of obesity [2]. Orlistat, a potent, specific, long-acting and reversible inhibitor of lipases, is a member of a new class of drugs available for the treatment of obesity. Orlistat plus diet has repeatedly demonstrated significantly greater weight loss when compared to the placebo plus diet. Moreover, the effects of orlistat are meaningful and meet the FDA standards of efficacy for prescription

weight control drugs [5]. The safety of orlistat is supported by a comprehensive body of data including preclinical animal testing and controlled clinical trials. In other clinical trials, 8.8% of patients treated with orlistat discontinued treatment due to adverse events. Of the 40 million users of orlistat worldwide, 13 cases of severe liver damage have been reported. Other side effects of using orlistat include frequent, oily bowel movements [6]. *H. sabdariffa* Linn, a tropical plant, belongs to the superorder Malvaceae. It is believed to originate from East Africa [7]. *H. sabdariffa* plants are cultivated and consumed as vegetables and tea, whereas other Hibiscus varieties are planted for the fibres they produce. It is called by different names like Roselle and Sorrel in English [8]. Various types of highly valued food and medicinal products are produced from parts of the *Hibiscus sabdariffa* including the seeds, leaves, fruits and roots. Among them, the fleshy red calyces are the most popular [9].

MATERIALS AND METHODS

Experimental animals- Twenty-eight (28) adult male albino rats, weighing between (100 - 220g) were used in this experiment during the period from 2019 to 2020 in the Department of Biochemistry, College Veterinary Medicine, Khartoum University. The rats were kept in a cage in the same environment with 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 routine clinical examination to ensure that they were healthy.

Feeding and watering of animals- The housing system was provided with appropriate facilities for feeding and watering. The nutritional regimen comprised HFD and ND. 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.

Experimental design- The distribution of the experimental rats into 4 groups, 7 rats. All groups were fed for six weeks, rats in group (A) where healthy control rats were fed on a normal diet (ND), obese rats in groups B, C and D received a high-fat diet (HFD), obese rats in group (C) were administered 12 mg/kg body weight of the oral intubation orlistat for 45 days and rats in group (D) were administered 200 mg/kg body weight of the *H. sabdariffa* for 45 days.

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

Ingredients	Normal Diet (ND)	High-Fat Diet (HFD) (%)
Protein (%)	18	10
Fat (%)	10	74.4
Carbohydrate (%)	66.4	10
Minerals (%)	3.5	3.5
Vitamin (%)	1	1
Methionine (%)	0.1	0.1
Fiber (%)	1	1
References	Pugh *et al.* [10]	Altunkaynak [11]

Orlistat drug- Purchased from Aljefn Pharmacy tries, Saudi Arabia as capsules each capsule contains 120 mg. Orlistat drug was marketed as a prescription under the trade name Xenical by Roche in most countries and is also known as tetrahydrolipstatin.

Hibiscus extract- Dried *Hibiscus sabdariffa* calyces were bought from the local market. Were weighed freshly every morning and they were dissolved, in distilled water at a dose of 200 mg/kg, manually shaken for 5 min and given orally, daily.

Body Weight Gain-Body weight for all rats in every group was recorded before study initiation (week 0) and at weeks 2, 4 and 6.

Biochemical analysis- Blood for sera was collected in plain containers from the retro-orbital plexus. Serum samples were kept at -20°C for biochemical analysis. All parameters were measured using commercial kits (BioSystem S.A. Costa Brava 30, 08030 Barcelona-Spain). The values obtained were read with a spectrophotometer (UV mini-1240 U.V./vis. Spectrophotometer, Japan) at appropriate wavelengths and the values were calculated using standard formulae [12].

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Data 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) [13]. p≤0.05 were considered statistically significant.

RESULTS

Body Weight (BW/g)- Table 2 shows the body weight of A, B, C and D groups. There was a significant (p-value= 0.003) increase in body weight in group (B) when compared to group (A). In treated groups, there was a significant decrease in groups (C and D) when compared to group (B).

Table 2: Effects of hibiscus treatments on body weight gain in obese rats (g)

Time	Group A	Group B	Group C	Group D
Week 0	125.7±10.78	168.4±23.85	185.7±22.88	160±19.84
Week 2	132.8±9.24	172±23.47	180.7±20.83	157±20.52
Week 4	134±9.53	179.1±19.12	172.6±19.81	152.6±19.76
Week 6	142.6±7.76	182.2±16.88	161±18.96	143.6±19.87

Serum biochemical changes

Serum Cholesterol-Generally, higher significant values (p-value= 0.000) of cholesterol were seen in group (B) when compared to the normal group (A) (Table 3). There was a significant (p-value= 0.001) reduction in cholesterol levels in treated groups when compared to group (B).

Table 3: Effects of hibiscus treatment on serum cholesterol level in obese rats (mg/dl)

Time	Group A	Group B	Group C	Group D
Week 0	119.5±16.1	179.2±12.2	168±16.87	162.11±7.12
Week 2	120.1±14.36	186.5±14.17	166.9±37.4	152.4±21.8
Week 4	118.7±3.29	190.9±16.36	155.5±10.2	145.38±9.15
Week 6	121.5±40.59	198.2±12.95*	147.9±4.16	141.4±3.22

Serum Aspartate Aminotransferase (AST)-Table 4 illustrates that serum AST enzyme values increased significantly (p-values= 0.025) in group B compared with group (A). However, there was a decline in serum AST enzymes in treated groups (C and D) when compared with group (B).

Table 4: Effects of hibiscus treatment on serum AST level in obese rats (U/L)

Time	Group A	Group B	Group C	Group D
Week 0	52.6±7.63	57.9±5.2	63.5±12.54	60.3±11
Week 2	55.3±6.63	55.5±5.82	61.3±5.13	55.3±5.2
Week 4	48.2±5.72	62.6±9.61	58.3±5.34	52.3±6.75
Week 6	43.9±6.54	64.6±5.13*	56.5±5.68	50.6±5.29

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Serum Alanine Aminotransferase (ALT)- Table 5 showed that serum ALT values increased significantly (p-values= 0.002) in group (B) as compared to group (A). Administration of Hibiscus (group D) resulted in the best ameliorating effect on serum ALP levels represented in significant decreasing effect compared with group (B).

Table 5: Effects of hibiscus treatment on serum ALT level in obese rats (U/L)

Time	Group A	Group B	Group C	Group D
Week 0	45.7±14.72	69.8±3.46	82.2±6.32	70.5±21
Week 2	46.6±10.06	70.4±10.99	79.1±10.81	68.3±9.04
Week 4	48.2±6.28	76.5±18.52	77.3±7.27	62.6±10.56
Week 6	50.7±5.7	81.2±3.06*	76.7±12.66	59.5±4.99

Serum Alkaline Phosphatase (ALP)- After six weeks the changes in the ALP of experimental animals were observed, the levels of ALP were significantly increased in group (B) (rats fed HFD) when compared with group (A) animals this was shown in Table 6. Group (C) (orlistat-treated rats) and group (D) (rats treated HSE) were found to have significantly decreased levels of ALP, this result was compared with group (B) animals.

Table 6: Effects of hibiscus treatment on serum ALP level in obese rats (U/L)

Time	Group A	Group B	Group C	Group D
Week 0	55.5±10.55	87.9±11.47	78.8±12.2	72.8±9.26
Week 2	54.6±28.19	85.9±7.68	77.2±25.79	70.5±6.77
Week 4	51.2±5.81	80.74±6.07	74.9±3.02	65.7±8.84
Week 6	57.5±2.65	89.9±11.45*	72.47±5.87	69.4±3.81

Serum Triglycerides- After six weeks the changes in the triglycerides of experimental animals were observed the levels of triglycerides were significantly increased in group B and were compared with group A animals. Group (C) and group D were found to have significantly decreased levels of triglycerides when compared to group (B) animals (Table 7).

Table 7: Effects of hibiscus treatment on serum Triglycerides level in obese rats (mg/dl)

Time	Group A	Group B	Group C	Group D
Week 0	98.9±28.15	151.4±32.82	171.24±26.39	143.77±30.28
Week 2	89.3±10.9	158.8±15.4	165.3±7.3	140.6±22.73
Week 4	95.2±14.9	161.5±4.3	163.1±11.7	139.8±66.9
Week 6	99.9±11.84	169.5±11.68*	159.8±18.65	136.3±26.39

Serum Urea- The levels of urea were significantly increased in group B animals when compared to group A and HSE-treated (group D) animals. In the case of orlistat treated (group C) showed significantly increased levels, when compared with group B as presented in Table 8.

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Table 8: Effects of hibiscus treatment on serum urea level in obese rats (mg/dl)

Time	Group A	Group B	Group C	Group D
Week 0	35.1±6.4	41.2±5.88	41.9±7.43	36.4±5.39
Week 2	38.8±5.67	44.43±11.66	41±7.57	37.4±5.34
Week 4	31.1±4.32	44.86±20.1	43.8±13.57	34.7±16.49
Week 6	33.2±6.38	45.55±6.01	50.17±13.8*	38.3±5.39

Serum low-density lipoprotein (LDL)- The levels of LDL were increased in rats fed with HFD (group B) when compared to the group (A) animals. The case of orlistat-treated (group C) and HSE-treated (group D) showed a significant decrease in levels when compared with group B as presented in Table 9.

Table 9: Effects of hibiscus treatments on serum LDL level in obese rats (g/dl)

Time	Group A	Group B	Group C	Group D
Week 0	111.9±57.76	128.4±13.9	126.61±11.22	120.1±23.6
Week 2	109.3±14.14	137.5±4.5	121.1±9.3	119.8±4.3
Week 4	110±37.8	138.39±9.36	123.1±16.7	118.09±9.15
Week 6	105.2±12.1	139.3±31.7	116.4±2.8	114.3±8.7

Serum high-density lipoprotein (HDL)-The levels of HDL were increased in rats fed with HFD (group B) when compared to the group (A) animals. In the case of orlistat treated (group C) and HSE treated (group D) showed significant decrease levels when compared with group B as presented in Table 10.

Table 10: Effects of hibiscus treatments on serum HDL level in obese rats (g/dl)

Time	Group A	Group B	Group C	Group D
Week 0	41.4±32.64	66.77±14.42	82.23±13.96	57.41±11.73
Week 2	39.5±2.6	59.7±2.8	75.9±3.1	46.3±3.3
Week 4	35.8±7.97	64.3±5.4	77.1±5.5	44.4±3.5
Week 6	47.2±4.4	68.6±12.6	79.1±6.34	58.2±2.6

Serum very low-density lipoprotein (VLDL)-The levels of VLDL were significantly increased in group B animals when compared to group A animals. In the case of HSE treated group (D) showed slightly decreased levels of VLDL, when compared with group C as presented in Table 11.

Table 11: Effects of hibiscus treatments on serum VLDL level in obese rats (g/dl).

Time	Group A	Group B	Group C	Group D
Week 0	19.78±5.63	30.28±6.56	34.25±5.28	28.75±6.05
Week 2	17.85±2.17	31.76±.08	33.06±1.45	28.13±4.54
Week 4	19.03±2.97	32.3±0.85	32.63±2.33	27.96±13.38
Week 6	19.98±2.36	33.9±2.33	31.96±3.73	27.25±5.27

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DISCUSSION

Obesity is a complex disease that gives rise to the interaction of dietary, a myriad of genetic, environmental factors and lifestyle which make a positive energy balance and lead to increased body fat mass [14]. Recent pharmacological approaches for treating and controlling obesity have resorted to the use of drugs to induce anorexia, inhibit nutritional absorption, and promote weight loss [15]. In our study, HSE were added to the rats, and tested for their ability to reduce body weight gain and prevent obesity.

In the present study, obese rats treated with HSE (group D) showed a significant decline in body weight when compared with obese control rats (group B). This result agreed with the result reported by Janson *et al.* [16]; Kao *et al.* [17]; and Alessandra *et al.* [18] were showed that the body weight gain was decreased in rats fed with a high-fat diet and treated with HSE when compared to the control rats. This is a similar result that was also reported by To-Wei *et al.* [19] showed that the body weight gain was decreased in hamsters fed with a high-fat diet and treated with HSE as compared to the obese hamster's control. Also, agreement with the result reported by María *et al.* [20] showed that the body weight was decreased in obese rats when treated with HSE when compared to the control rats. The loss in BW may be due to *H. sabdariffa* inhibiting the activity of a-amylase, blocking sugars and starch absorption, which may assist in weight loss [21,22].

In the present study, the increase of serum cholesterol levels in HFD rats (group B) may enhance the risk of fatty liver and atherosclerosis [23]. Obese rats treated with HSE (group D) showed a decrease in serum cholesterol when compared with rats who received HFD (group B). This result agreed with the result reported by Aljamal [24]; and Chukwu *et al.* [25] who showed that the serum cholesterol was reduced in rats fed with high-fat diets when treated with HSE when compared with control rats. Also, a similar result was reported by Nnamonu *et al.* [26] who reported that the level of cholesterol was decreased in rats fed with a high-fat diet and treated with HSE. This result disagreed with the result reported by Francisco *et al.* [27] showed that the cholesterol was increased in obese rats treated with HSE when compared to the control rats. This hypocholesterolemic may be attributed to the abundant antioxidant composition of *H. sabdariffa* especially anthocyanins [28].

In the present study, obese rats treated with HSE (group D) showed a significant decrease in AST and ALT when compared with rats who received HFD (group B). This result agreed with the result reported by Kao *et al.* [17]; and Dahiru *et al.* [29]. They suggested that the AST and ALT serum levels were reduced in albino rats treated with HSE, a similar result was also reported by To-Wei *et al.* [19] who showed that the level of AST and ALT declined in obese hamsters treated either with HSE. Furthermore, identical results reported by Nnamonu *et al.* [26] showed that the levels of AST and ALT were decreased in rats treated with HSE. This result disagreed with the result reported by Francisco *et al.* [27] showed that the ALT was increased in obese rats treated with HSE when compared to the control rats. The reduction of liver enzymes after HSE treatment indicated a decrease in fat deposition and necrosis in liver cells [30].

In the present study, obese rats treated with HSE (group D) showed a significant decrease in ALP when compared with rats that received HFD (group B) this result agreed with the result reported by Nnamonu *et al.* [26] They showed that the ALP was reduced in rats treated with HSE when compared to the control rats. This similar result was also reported by Ebhohon *et al.* [31] who showed that the level of ALP declined in obese rats treated with HSE**.** Treatment with HSE noticeably improved liver function by decreasing enzyme markers like ALP levels which proved an important role of hibiscus in preventing liver damage.

The present study of obese rats treated with HSE (group D) showed a turndown in serum triglycerides when compared with rats that received HFD (group B) this result agreed with the result reported by Janson *et al.* [16] they showed that the serum triglycerides were reduced in rats fed with high-fat diet when treated with HSE. Also, a similar result was reported by Showande *et al.*[32] who showed that the level of triglycerides declined in rats treated with HSE**.** Also, this result agreed with the result reported by Mohammad *et al.* [33] which showed that the level of triglycerides declined in obese adolescents treated with HSE. This result disagreed with the result reported by Francisco *et al.* [27] showed that the triglycerides were increased in obese rats treated with HSE when compared to the control rats. The decrease of triglycerides may be due to the lipase

activity being blocked, triglycerides from the diet are not hydrolyzed into absorbable free fatty acids and are excreted undigested instead [34]. Also, the decrease in serum TG could be associated with lipid absorption inhibition in the gut [19]. Dietary lipids are absorbed into the bloodstream and are then digested to be transported and stored in the liver and adipose tissues in the form of TG [35].

In the present study, obese rats treated with HSE (group D) showed a significant decline in urea when compared with rats that received HFD (group B), this result agreed with the result reported by Alessandra *et al.* [18] showed that the urea was decreased in obese rats treated with HSE. The decrease of urea may be due to the protective effect of Hibiscus was associated with lipidic profile improvement, as well as correction of a marked reduction in glomerular filtration rate [18].

In the present study, obese rats treated with HSE showed a significant decrease in LDL when compared with rats who received HFD. This result could be due to fatty acids which are the building block for lipids are susceptible to the oxidation process partly through the presence of free radicals as intermediate [36] this result agreed with the result reported by Alessandra *et al.* [18] who showed that the LDL were reduced in obese rats treated with HSE when compared to the control rats. This similar result was also reported by Janson *et al.* [16] who showed that the level of LDL was decreased in rats fed with high-fat diet and treated with HSE. Also, this result agreed with the result reported by Mohammad *et al.* [33] who showed that the level of LDL declined in obese adolescents treated with HSE. This result disagreed with the result reported by Diantini *et al.* [37] who showed that the LDL was increased in humans treated with HSE when compared to the control.

In the present study, obese rats treated with HSE (group D) showed a decrease in HDL when compared with rats received HFD (group B) this result was similar result was reported by Umoren *et al.* [38] who showed that the level of HDL was decreased in albino rats treated with HSE. This similar result was also reported by Yusni and Meutia [39] who showed that the level of HDL was decreased in women treated with HSE. On the other side, this result disagreed with the result reported by Janson *et al.* [16]; Alessandra *et al*. [18] showed that the HDL was increased in obese rats fed with a high-fat diet and treated with HSE when compared to the control rats. The decrease in HDL may be due to the polyphenols, anthocyanins, and flavonoids in *H. sabdariffa.*

In the present study, obese rats treated with hibiscus showed a significant decrease in VLDL when compared with rats who received HFD (group B). This result agreed with the result reported by *Diantini et al*. [37] showed that the VLDL were reduced in humans treated with HSE when compared to the control. Also, a similar result was reported by Umoren *et al.* [38] who showed that the level of VLDL was decreased in rats treated with HSE when compared to the control rats. The decrease in VLDL may be due to attributed to the effects of the extract's bioactive compounds in augmentation of satiety, suppression of absorption and digestion of dietary lipids as well as inhibition of pancreatic lipase activity [39-41].

CONCLUSIONS

Obese rats treated with *Hibiscus sabdariffa* extract obtained a significant decrease in body weight, serum cholesterol, ALT, AST, TG and LDL when compared with the control 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. The study showed that there is a high need to perform a further study regarding the effect of *H. sabdariffa* extract to prefend the obesity and lipid profile on biochemical profile and decrease body weight in albino rats in Sudan. More studies are vital to determine the effects of the action of *Hibiscus sabdariffa* extract in obese rats. The study was to determine the effect of *H. sabdariffa* on body weight and lipid profiles in obese rats.

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CONTRIBUTION OF AUTHORS

Research concept: Dr. Selma E. Salih
Research design: Prof. Shamseldein H. Ahmed, Dr. Selma E. Salih
Supervision: Prof. Shamseldein H. Ahmed
Materials: Islam A. Daffalla
Data collection: Islam A. Daffalla, Dr. Selma E. Salih,
Data analysis and Interpretation: Islam A.Daffalla, Dr. Selma E.Salih
Literature search: Islam A.Daffalla

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Writing article: Islam A. Daffalla, Dr. Selma E.Salih
Critical review: Islam A. Daffalla, Dr. Selma E. Salih
Article editing: Islam A. Daffalla, Dr. Selma E. Salih
Final approval: Prof. Shamseldein H. Ahmed, Dr. Selma

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