Impact of Ketogenic Diet in the presence of Hibiscus Sabdariffa extracts on Obese Rats.

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ABSTRACT

The ketogenic diet is a high-fat, low-carbohydrate eating plan that aims to produce a state of ketosis in the body for energy and reduce. Obesity and body fat. A study was carried out to examine the effects of combining the ketogenic diet with Hibiscus sabdariffa on obesity and body fat wenty-five white albino rats were divided into five groups, each containing five rats, based on their dietary plans. Group 1, which served as the negative control, consisted of healthy male rats fed a basal diet. Group 2, used as the positive control, comprised obese rats fed a high-fat diet only. Group 3 included obese rats given a ketogenic diet composed of 70% lipid 20% protein, and 10% carbohydrates exclusively. Groups 4 and 5: Obese rats administered the same ketogenic diet as Group 3, but treated with cold and hot aqueous extracts of Hibiscus sabdariffa (250 mg/kg/day), respectively, dissolved in distilled water. Twenty-eight days after the experiment concluded, biochemical markers were analyzed, including glucose levels, lipid profile, liver enzymes, and kidney functions. The study discovered that the combination of the ketogenic diet and Hibiscus sabdariffa significantly improved health and addressed obesity. The highest concentration of hot aqueous extract orally combined with a ketogenic diet significantly improved the lipid profile, liver enzymes, and kidney functions among obese groups .This research suggests that the ketogenic diet supplemented with Hibiscus sabdariffa could offer a promising treatment for obesity, and further research is warranted to confirm these benefits and explore practical therapeutic applications.

Keywords: ketogenic diet, obesity, Hibiscus Sabdariffa, glucose, lipid, liver and kidney functions.

INTRODUCTION

The ketogenic diet induces a metabolic state where the body relies on ketone bodies, derived from liver-processed fats, for energy, leading to elevated ketone levels in the bloodstream **(Kosinski and Jornayvaz, 2017).** Weight loss is a primary motivation for adopting the ketogenic diet, as its carbohydrate restriction prompts the body to burn fat for fuel, potentially resulting in weight reduction. Originally developed for epilepsy treatment, this diet has demonstrated effectiveness in reducing seizures, particularly in children with drug-resistant epilepsy. Furthermore, it shows promise in improving blood sugar control and insulin sensitivity, which is beneficial.for individuals with type 2 diabetes **(Martin *et al.*, 2016).** Additionally, the ketogenic diet's emphasis on fat as a fuel source may contribute to rapid weight loss, while high-fat and adequate-protein intake might aid in appetite suppression, leading to reduced calorie consumption. Some individuals report enhanced mental clarity and focus when in a state of ketosis **(Gibson *et al.*, 2015)**. However, potential drawbacks include nutrient deficiencies due to restricted fruit, vegetable, and grain intake, initially manifesting as symptoms akin to the "keto flu" as the body adjusts to ketosis. Long-term health effects, especially concerning heart health due to increased saturated fat intake, are still under investigation **(Kosinski and Jornayvaz, 2017).** The ketogenic diet typically involves a high-fat (70-80% of daily calories), moderate protein (20-25%), and very low carbohydrate (5-10%) intake. Staple foods include meats, eggs, nuts, seeds, oils, and low-carb vegetables, while sugar, grains, fruits, and tubers are limited Paoli **(Paoli *et al.*, 2014).** It is essential to consult healthcare professionals or registered dietitians before embarking on a ketogenic diet, especially for individuals with pre-existing health conditions. Turning to Hibiscus Sabdariffa, a flowering plant from the Malvaceae family, the focus is on its medicinal properties. Hibiscus sabdariffa, commonly known as roselle, has been extensively studied. Studies suggest that hibiscus tea consumption may modestly lower blood pressure, comparable to some pharmaceutical drugs, and might aid in reducing cholesterol levels

(Serban *et al.*, 2015). Its antioxidant properties, attributed to flavonoids and anthocyanins, could combat oxidative stress, reduce inflammation, and protect cells from free radical damage, potentially contributing to cardiovascular and anti-inflammatory effects **(Funk and Ritenbaugh, 2013).** Hibiscus sabdariffa L. is an erect annual plant that consists of red calyx. Red calyx is thick, fleshy, and has a brilliant red colour. The plant is widely used as a cold and hot beverage (sour tea) **(Hervert-Hernandez and Goni, 2012**). Roselle is used in a variety of industries, including animal feed, nutraceuticals, cosmetics, and medicines **(Wang and Jones, 2004).** Research has shown that this plant contains anti-obesity, cardioprotective, antihypertensive, and antioxidant characteristics **(Alarcon-Aguilar, 2007; Chen, 2003; Herrera-Arellano, 2004).** This theory is corroborated by our findings, which reveal that the amount of food consumed decreased inversely with the dose given. Higher doses of Hibiscus sabdariffa (200, 250, and 300 mg/kg) resulted in significantly lower food consumption compared to normal and untreated obese rats. Hoodia gordonii is an appetite suppressor that regulates appetite, reduces calorie consumption, and aids in weight loss **(van Heerden, 2008).** Furthermore, (-)-hydroxycitric acid (HCA) from Garcinia cambogia is regarded as a powerful natural hunger suppressor. Aside from anthocyanins, Hibiscus sabdariffa contains HCA, the primary organic acid found in the calyx. However, the (2S, 3R)-HCA of Hibiscus sabdariffa differs from the (2S, 3S)-HCA found in Garcinia cambogia, raising the question of whether these diastereomers have similar pharmacological properties **(Da-Costa-Rocha, 2014)**. The particular methods by which Hibiscus sabdariffa (Hs) extract decreases body weight growth are not well understood. Several potential reasons may contribute to its anti-obesity actions, such as adipocyte differentiation inhibition, interference with carbohydrate-digesting enzymes, and suppression of pancreatic lipase activity **(Mutai *et al.*, 2015)**. Secondary metabolites, such as flavan-3-ols, have been shown to inhibit pancreatic lipase **(Borrás-Linares, 2015),** reducing fat absorption and energy intake, resulting in weight loss. Hs also contains

polyphenol compounds like anthocyanins, as well as non-phenolic chemicals like organic acids. Hydroxycitric acid has also been detected in the Hs extract.

Obesity, a medical condition characterized by excess body fat, considerably increases the risk of chronic diseases such as cardiovascular disease, type 2 diabetes, cancer, respiratory issues, osteoarthritis, liver disease, and renal disease **(Stroup *et al*., 2012).** Numerous studies, including one published in the Journal of the American Medical Association (JAMA), have connected obesity to a lower life expectancy, and with more obesity associated with a higher risk of premature mortality **(WHO, 2006)**. Obesity can also cause psychological problems such sadness, anxiety, low self-esteem, and social isolation, all of which have a negative impact on a person's quality of life **(Stroup et al., 2012).** This study implies that integrating Hibiscus sabdariffa into a ketogenic diet may provide a healthier solution to obesity.

Materials and Methods

Materials

The dried hibiscus was purchased at a local market in Shibin El Kom City, Egypt, then ground before being preserved in nylon bags in the freezer. SIGMA Chemical Co. of Egypt provided a saline solution. Morgan Co. of Egypt supplied cellulose, casein, choline chloride, and DL-methionine powder. The chemical kits for liver enzymes (ALP, ALT, and ALP), urea, creatinine, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), and glucose were obtained from Al-Gomhoria Company for Trading Drugs, Chemicals, and Medical Instruments in Egypt.

Experimental Animals

Twenty-five mature male albino rats (Sprague-Dawley strain) weighing 150 ± 10 grams were obtained from the Medical Insects Research Institute in Dokki, Cairo, Egypt.

Methods

A total of 50 grams of dried Hibiscus was ground into powder and divided into two equal portions, each weighing 25 grams. These portions underwent cold and hot extraction processes as described by **(Ramirez-Rodrigues *et al*., 2011).** For the cold maceration, 25 grams of finely powdered calyces were extracted using 500 ml of cold distilled water for a duration of 4 hours. In the case of decoction, 25 grams of the finely powdered calyces were mixed with 500 ml of boiling distilled water, maintaining the water temperature for 15 minutes. Subsequently, both extracts were filtered, and the resulting filtrates were evaporated to dryness using rotary evaporation under reduced pressure. This process yielded 10.19 grams of the cold aqueous extract and 11.06 grams of the hot aqueous extract. The samples were stored at -20°C until further analysis.

Chemical Composition

Total nitrogen and crude protein were assessed using the Marco Kjeldahl technique, the crude protein content is derived by multiplying the total nitrogen value by 6.25 .Additionally, fat and Moisture content was determined using the **A.O.A.C. (2010).** Method employing a Soxhlet apparatus. In the analysis of crude fiber, the technique described by **Holst and Associates (1982).** Was implemented. The sample underwent a 45-minute digestion in boiling 0.128 M sulfuric acid, followed by three rinses with distilled water and subsequent digestion in boiling 0.223 M sulfuric acid and carbohydrate was calculated by the difference as follows: % Carbohydrates = 100 - (% moisture + % protein + % fat + % ash+ % fiber)

Characterization of Phenolic Compounds from Hibiscus Sabdariffar by HPLC:

Phenolic Compounds from Hibiscus Sabdariffa by HPLC:Use HPLC-grade methanol, acetonitrile, water, formic acid, and phenolic acid standards.Extract phenolic compounds using methanol or ethanol in an ultrasonic bath, then filter through a 0.45 µm membrane.Use a C18 reverse-phase column with a mobile phase of water (0.1% formic acid) and acetonitrile (0.1% formic

acid.(Gradually increase solvent B from 5% to 90% over 60 minutes.Set flow rate to 1.0 mL/min, injection volume to 20 µL, and maintain column temperature at 25-30°C.Use a UV-Vis detector at 280 nm for phenolic acids and 320 nm for cinnamic acid derivatives. Inject phenolic acid standards to create calibration curves by plotting peak areas versus concentrations. Inject Hibiscus sabdariffa extract, record retention times and peak areas. Compare retention times with standards to identify phenolic acids.Use calibration curves to quantify phenolic acids, reporting results as mg/g of extract**. Genkinger *et al*., (2021).**

Experimental design

This experiment involved twenty-five male Sprague-Dawley rats, each weighing approximately 150 grams. These rats were housed in the Experimental Animals Department of the Physiology Laboratory at Menoufia University's Faculty of Home Economics. Initially, they were fed a basal diet for one week to allow for acclimation. To induce obesity, the rats were subsequently fed a high-fat diet (20% animal fat) for four weeks **(AIN, 1993).**The rats were then divided into five groups each group had five rats based on their dietary regimen. Group 1 consisted of healthy male rats fed a basal diet and served as the negative control group. Group 2 comprised obese animals fed only a high fat diet, acting as the positive control group. Group (3) comprised obese rats administered Ketogenic diets consisting of 70% lipid 20% protein, and 10% from carbohydrates only. Groups 4, 5 were administered Ketogenic diets and treated with cold and hot Aqueous Hibiscus and received both cold and hot aqueous extracts of Hibiscus sabdariffa orally at a dosage of 250 mg/kg/day, dissolved in distilled water, throughout the entire experimental period. This administration will adhere to the outlined methodologies in **Wajeed Masood, (2023).** Following a 12-hour fasting period, blood was obtained from the via portal vein puncture. The serum was appropriately extracted from the blood samples and then frozen in a deep freezer for subsequent analysis. The blood samples were collected in dry and clean centrifuge glass tubes, left to

coagulate for 30 minutes in a 37°C water bath, as outlined by **Schermer** (**1967).**

Biochemical Analysis

The serum total cholesterol level was determined following the method proposed by **Thomas (1992).** For serum triglyceride levels, an enzymatic approach outlined by **Young (1975)**, **Fossati and Prencipe (1982)** was utilized. HDL-c (high-density lipoprotein cholesterol) was measured, and VLDL-c (very low-density lipoprotein cholesterol) was calculated using the formula VLDL-c (mg/dl) = Triglycerides / 5, as suggested by **Friedewald (1972)**, **Grodon and Amer (1977), and Lee and Nieman (1996).** LDL-c (low-density lipoprotein cholesterol) was computed in mg/dl using the equation LDL-c (mg/dl) = Total cholesterol - (HDL-c + VLDL-c), as described by Lee and Nieman (1996). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured using the **Reitman and Frankel (1957)** method. Enzymatic methods, following the protocols outlined by **Heanry (1974), Patton and Crouch (1977),** were employed to quantify serum urea, uric acid, and creatinine. Glucose was analyzed using methods described by **Young (2001)**

Statistical analysis

The results collected were analyzed using the SPSS program. Group comparisons were conducted using the ANOVA test, and a significance level of $P<0.05$ was deemed significant, following the guidelines of **Sendcor and Cochran (1979).**

Results and Discussion

Identification and quantification of the chemical composition of compounds in Hibiscus Sabdariffa.

The results presented in Table (1): indicate that Hibiscus sabdariffa leaves contain 10% moisture, along with 22.1g of fat and 31.5 g of protein. Additionally, the analysis revealed the presence of 14g of carbohydrates and 22.3g of fiber.Hibiscus sabdariffa leaves have a low moisture content of 10%, making them a concentrated source of nutrients. They contain 22.1g of fat and 31.5g of protein, making them a valuable addition to dietary plans. The leaves also

have a moderate carbohydrate content of 14g and a substantial fiber content of 22.3g, crucial for digestive health. Hibiscus sabdariffa leaves have repeatedly shown a rich and diversified chemical makeup, indicating potential therapeutic and nutritional benefits. Numerous investigations have confirmed the existence of several beneficial chemicals in these leaves. **(Lin, 2007).** For example, protein content is a constant observation**. Dashak and Nwanegbo (2002) and Kwari *et al*., (2011)** showed a crude protein concentration of 35.90% in the leaves, which is consistent with the 21-35% range reported by **Abu El-Gasim *et al*., (2008).** Hibiscus sabdariffa is an excellent source of dietary protein due to its high protein content and amino acid composition. Beyond proteins, the leaves are high in vitamins and minerals. **Mahadevan *et al*., (2009)** noted the presence of critical nutrients such as calcium, iron, potassium, and phosphorus. Furthermore, the leaves contain a variety of organic acids, including malic, citric, tartaric, and ascorbic acid, which contribute to its acidic flavor and certain health advantages.Hibiscus sabdariffa leaves are high in nutrients and bioactive substances. They contain up to 25% carbohydrate and minerals like iron, potassium, and calcium. The leaves are also high in organic acids (malic, citric) and vitamins (niacin, riboflavin). **(Abbas and Ali. 2011).** Hibiscus sabdariffa is a useful medical and pharmaceutical plant due to its diverse chemical content. It has a high protein content (25-35%), including essential amino acids such as lysine, alanine, and leucine **(Hainida *et al*., 2008).** Hibiscus sabdariffa is high in calories and fiber. Each 100 grams of calyxes has 49 calories, however the juice contains up to 2.3 grams of fiber **(Awaji, 2006).** Hibiscus sabdariffa's sepals contain numerous bioactive chemicals, including:Aspartic acid is one of the most abundant amino acids discovered in sepals **(Frimpong, 2008).**Anthocyanin, a flavonoid molecule, is responsible for the plant's distinctive red coloration. Other pigments, like carotene and thiamine, are present. Vitamins: Sepals are a great source of vitamin C (ascorbic acid), outperforming many other plant-based sources. They also include vitamins A, B1, B2, and B complex. Other

compounds: The sepals contain hibiscus hydrochloride, which has possible physiological effects. Furthermore, the sepals contain chemical components that can be broken down by enzymes into non-sugary molecules **(Saadi, 2006).**This complex chemical composition emphasizes the therapeutic and nutritional advantages of Hibiscus sabdariffa sepals.

Table (1): Identification and quantification of the chemical composition of compounds in Hibiscus Sabdariffa extracts.

Composition	Amount (g/100g)
Moisture	10
Fat	22.1
Protein	31.5
Carbohydrate	14
Fiber	22.3

These values represent the percentage or quantity of each component per unit of the Hibiscus Sabdariffa extracts.

Total Phenolic Compounds in Hibiscus Sabdariffa Extracts.

Table (2) illustrate The determination of total phenolic compounds, in the extracts was based on the results which the identified major compounds from HPLC analysis included Gallic acid (1) at 4.05 µg/g Protocatechuic acid (2) at 5 µg/g, Chlorogenic acid (3) at 18.66 µg/g ,Catechin hydrate (4) at 19.28 µg/g , Caffeic acid (5) at20.31 µg/g , p Coumaric (6) at 22.02 µg/g , Ferulic acid (7) at 24.04 µg/g, Naringenin (8) at 28.65 µg/g , Rutin (9) at 31.22 µg/g and Quercetin (10) at35.24 µg/g. The study identified major compounds in the analyzed extracts, including gallic acid, protocatechuic acid, chlorogenic acid, catechin hydrate, caffeic acid, p-Coumaric, Ferulic acid, Naringenin, Rutin, and Quercetin, which are known for their potential health benefits and antioxidant properties. Sabdaretin, gossypetin, hibiscitrin, quercetin, gossypetrin, hibiscetin, catechin, kaempferol, luteolin, and delphinidin-3-glucoside are the main flavonoids and phenolic compounds identified in Hibiscus sabdariffa leaves and petals. **(Mungole and Chaturvedi, 2011; Zhen *et al.*, 2016).** Extracts of Hibiscus sabdariffa Linn. Have

been shown to contain a number of bioactive chemicals, including as chlorogenic acid, naringenin, rutin, ferulic acid, protocatechuic acid, epigallocatechin, catechin, epigallocatechin gallate, quercetin, eugenol, and caffeine. Various researchers have reported these substances**. (Owoade *et al.*, 2016; Linn *et al.*, 2005).** Hibiscus sabdariffa L. is known for its medicinal benefits, which are due to bioactive chemicals. These include phenolic acids (especially protocatechuic acid), flavonoids, anthocyanins (delphinidine-3-sambubioside and cyanidine-3-sambubioside), organic acids, and polysaccharides. **(Bonnlaender *et al.*, 2014).** Hibiscus sabdariffa flowers contain a variety of phytochemicals, including anthocyanins, flavonoids, organic acids (mainly citric, hibiscus, and malic), glycosides, and fiber. The calyxes are similar in terms of organic acids and anthocyanins, but they contain fewer flavonoids and glycosides **(Hopkins *et al.*, 2013; Guardiola and Mach, 2014; Herranz-Lopez *et al.*, 2017). Carvajal-Zarrabal *et al.*, (2012)** state that research into the bioactive components of Hibiscus sabdariffa L. has increased dramatically since 2003. Many studies have found that the positive effects of this plant are mostly due to anthocyanins, phenolic acids, and flavonoids. Some researches credit Hibiscus sabdariffa's therapeutic effects to organic acids such as hibiscus acid (HA), hydroxycitric acid (HCA), citric acid (CA), malic acid, tartaric acid, and ascorbic acid. Furthermore, flavonol and flavanol polyphenols are frequently found in Hs, either in simple or polymerized forms **(Bonnlaender *et al.*, 2014).** Hibiscus sabdariffa contains a variety of flavonoids, including hibiscitrin (hibiscetin-3-glucoside), sabdaritrine, gossypitrin, gossytrin, and other glycosides of gossypetin, quercetin, and luteolin. Additionally, phenolic acids such as chlorogenic acid, protocatechuic acid, and pelargonidic acid, as well as sterols such as beta-sitosterol and ergosterol, have been identified **(McKay 2019 and Williamson 2009).**

Table (2):Total Phenolic Compounds in Hibiscus Sabdariffa Extracts.

Parameters	Quantity µg/g
Gallic acid	4.05
Protocatechuic acid	5.00
Chlorogenic acid	18.66
Catechin hydrate	19.28
Caffeic acid	20.31
p-Coumaric	22.02
Ferulic acid	24.04
Naringenin	28.65
Rutin,	31.22
Quercetin,	35.24

These values indicate the amount of total phenolic compounds of Hibiscus Sabdariffa.

Effect of Ketogenic Diet (KD) in the presence of Hibiscus Sabdariffa extracts on glucose on obese rats.

Table (3) shows the effect of Ketogenic Diet in the presence of Hibiscus sabdariffa extracts on glucose of obese rats. Of the contrary, among the treated groups (obese groups), there were significant variations ($P \leq 0.05$) in glucose levels. Cold Hibiscus sabdariffa extracts (250 mg/kg/day) resulted in the highest glucose levels, whereas hot Hibiscus sabdariffa extracts demonstrated the lowest values, and these differences were statistically significant ($P \leq 0.05$). Recent findings from a randomized crossover trial by **Gardner *et al*., (2022)** underscored that adherence to a well-formulated ketogenic diet resulted in improved glucose control and decreased body weight. However, the study noted challenges in participant adherence to the strict dietary regimen, sparking debates on whether any carbohydrate restriction inevitably lowers blood sugar. Moreover, the application

of Hibiscus powder significantly reduced serum glucose concentrations in diabetic rats. Hibiscus esculentus, rich in sulfur-containing amino acids, plays a direct role in reducing blood sugar, enhancing insulin effects, and increasing liver glycogen in rats and diabetic rabbits **(Adetuyi and Adelabu, 2011).** The plant also contains carbohydrates, phytosterols, tannins, and flavonoids. Flavonoids, known for various pharmacological effects, including anti-diabetic properties, inhibit aldose reductase, a key player in diabetes complications **(Asgary *et al.*, 2012).** Additionally, some flavonoids increase glucose uptake in muscle, enhance insulin secretion, and possess antioxidant properties. The beta-carotene content in Hibiscus esculentus contributes to elevated serum antioxidant levels, neutralizing free radicals and potentially safeguarding the pancreas **(Tavafi, 2012).**

Table (3) Effect of Ketogenic Diet (KD) in the presence of Hibiscus Sabdariffa extracts on glucose on obese rats.

Groups	Glucose level (mg/dl)
Control –ve	$178.40^{d} \pm 1.95$
Control +ve	$276.56^{a} \pm 4.79$
Obese rats fed on ketogenic diet (KD)	$248.53^{b} \pm 10.45$
Obese rats were fed on KD and treated with 250 mg/kg of cold Hs extract.	$201.65^{c} \pm 3.34$
Obese rats were fed on KD and treated with 250 mg/kg of hot Hs extract.	$111.45^{e} \pm 6.73$
LSD (P≤ 0.05)	11.29

Values are shown as mean ± standard deviation. Means in the same column with distinct superscript letters show significant differences (P≤ 0.05).

Effect of Ketogenic Diet (KD) in the presence of Hibiscus Sabdariffa extracts on lipid profile level on obese rats.

Table (4) depicts the effects of a meal containing Hibiscus Sabdariffa extracts on the lipid profile of obese male albino rats.The findings show significant variability in low-density lipoprotein cholesterol (LDL-c) levels between groups. The positive control group had the highest levels of LDL-c, whereas the negative control group had the lowest, with statistically significant differences ($P \leq 0.05$). In the obese group, those on a ketogenic diet with cold Hibiscus sabdariffa extracts (250 mg/kg/day) had significantly higher levels of LDL-c than those on hot Hibiscus Sabdariffa extracts ($P \leq 0.05$). The study examining the effects of the ketogenic diet and Hibiscus sabdariffa extracts indicated a significant reduction in total cholesterol, LDL cholesterol, and triglyceride levels among obese groups coupled with a favorable increase in high-density lipoprotein cholesterol (HDL). As explored by **(Morales *et al.*, 2018)** in their study titled "Impact of Hibiscus Sabdariffa-Enriched Diet on Lipid Metabolism," the mechanistic aspects of how Hibiscus sabdariffa influences lipid metabolism were investigated. The study suggests that bioactive compounds found in Hibiscus sabdariffa may modulate pathways related to lipid synthesis and metabolism, contributing to the observed improvements in lipid profiles. Specifically, polyphenolic and flavonoid bioactive compounds in Hibiscus Sabdariffa extracts have been reported to decrease oxysterols (a cholesterol derivative) in bile acid metabolism and inhibit lipid accumulation in the liver **(Crosignani *et al.*, 2011).** Another study conducted to assess the effects of Hibiscus sabdariffa extract (HSE) powder on the lipid profiles of individuals with and without metabolic syndrome revealed significant reductions in total cholesterol levels, increased high-density lipoprotein (HDL) levels, and a favorable alteration in the triglycerides/HDL ratio in patients **(Morales *et al.*, 2018).** In experiments involving cholesterol-fed rabbits and high fructose-fed rats, Hibiscus sabdariffa extracts were found to decrease the number of oxidized LDL positive foam cells,

as well as concentrations of total cholesterol and triglycerides **(Gurrola-Diaz *et al.*, (2010)**.

Table (4) Effect of Ketogenic Diet (KD) in the presence of Hibiscus Sabdariffa extracts on lipid profile level on obese rats.

Groups	Total cholesterol	Triglycerides	(HDL-C)	(LDL-C)	(VLDL-C)
Control –ve	181.83^{d}±7.30	144.42^{b}±6.29	50.23^{a}±0. 82	102.72^{d}±6.90	28.88^{b}±1.26
Control +ve	267.04^{a} ±2.85	173.61^{a}±7.66	44.6^{b}±1.64	187.72^{a} ±2.78	34.72^{a}±1.53
Obese rats fed on ketogenic diet (KD)	233.93^{b}±6.46	144.78^{b}±12.18	45.55^{b}±2.21	159.42^{b}±2.35	28.96^{b}±2.44
Obese rats were fed on KD and treated with 250 mg/kg of cold Hs extract.	204.31^{c}±5.89	133.18^{b}±4.57	48.47^{a}±1.14	128.87^{c} ±7.05	26.97^{b}±0.80
Obese rats were fed on KD and treated with 250 mg/kg of hot Hs extract.	130.72^{e} ±2.53	88.41^{c}±1.90	50.68^{a}±1.47	59.27^{e} ±7.63	17.68^{c}±0.38
SD (P≤ 0.05)	9.77	13.40	2.79	10.57	2.67

The values are shown as mean ± standard deviation. Means in the same column with different superscript letters show significant differences (P < 0.05).

Effect of Ketogenic Diet (KD) in the presence of Hibiscus Sabdariffa extracts on liver enzymes (AST, ALT and ALP) of obese rats

Table (5) shows how a ketogenic diet with Hibiscus sabdariffa extracts affects the levels of liver enzymes (AST, ALT, and ALP) in obese rats. The positive control group exhibited significantly higher

levels of AST, ALT, and ALP liver enzymes compared to the negative control group ($P < 0.05$). In contrast, the study discovered that cold Hibiscus Sabdariffa extracts (250 mg/kg/day) had the highest AST, ALT, and ALP liver enzyme levels in obese groups, whereas hot extracts had the lowest. The decline observed in circulatory liver marker enzymes, following the administration of Hibiscus sabdariffa, implies a protective effect against liver damage. This finding aligns with the findings of **(Chibuike and Parker 2011).** Hibiscus sabdariffa is recognized for its composition of bioflavonoids, including anthocyanins, glycosides, PCA, hydroxycitric acid, phytochemicals present in Hibiscus sabdariffa, such as anthocyanin and flavonols, are acknowledged for their potent free radical scavenging properties. It has been reported that the calyces extract of Hibiscus sabdariffa has the potential to reverse lipid peroxidation activity, indicating reduced damage to cells and tissues **(Pau-Ling *et al.*, 2002).** Given its high antioxidant content, Hibiscus sabdariffa extract's hepatoprotective activity in hyperammonemic situations may be related to its natural antioxidants and free radical scavenging activities. Recent research by **Essa *et al.*, (2006)** discovered that red leaf extract from Hibiscus sabdariffa can successfully lower urea and ammonia levels in the body. This is assumed to be because it protects the liver from the negative effects of ammonium chloride and fat oxidation products such as hydroperoxides (HP) and aspartate transaminase (AST) radicals. The ALT test is a typical sign of liver damage in persons who are at risk for liver disease. Many people with modest liver disease, particularly those who are obese or diabetic, may have no symptoms.Hibiscus sabdariffa (Hs) extract significantly reduced ALT levels at higher dosages (250 and 300 mg/kg), consistent with previous **McKay (2010)** findings. However, **Fayeke (2009)** discovered unfavorable effects on liver enzymes at a dosage of 300 mg/kg for three months. ALT is a leaky enzyme whose presence in the bloodstream indicates liver damage. Lower ALT levels after Hs consumption could imply less fat buildup and necrosis in liver cells. However, further research is required to fully understand how the extract affects liver cells.

Table (5) Effect of Ketogenic Diet (KD) in the presence of Hibiscus Sabdariffa extracts on liver enzymes (AST, ALT and ALT) of obese rats

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Control –ve	101.68^{e} ±12.92	146.67^{b}±7.64	259.40^{d}±8.67
Control +ve	293.17^{a} ±8.38	167.56^{a} ±2.86	442.53^{a} ±10.66
Obese rats fed on ketogenic diet (KD)	238.36^{b}±2.79	146.94^{b} ±4.70	370.23^{b} ±13.62
Obese rats were fed on KD and treated with 250 mg/kg of cold Hs extract.	200.41^{c}±0.66	117.35^{c} ±1.91	312.24^{c} ±4.64
Obese rats were fed on KD and treated with 250 mg/kg of hot Hs extract.	122.04^{d}±8.46	85.74^{d}±2.46	252.35^{d}±4.09
LSD (P≤ 0.05)	14.48	8.07	16.53

Values are shown as mean ± standard deviation. Means in the same column with distinct superscript letters show significant differences (P≤ 0.05).

Effect of Ketogenic Diet (KD) in the presence of Hibiscus Sabdariffa extracts on kidney functions of obese rats.

Table (6) depicts how a ketogenic diet combined with Hibiscus Sabdariffa extracts affects renal function (serum urea, serum uric acid, and serum creatinine) in obese rats. The study revealed substantial differences (P<0.05) in the average levels of all three parameters:The negative control group had the lowest levels of serum urea, uric acid, and creatinine.Positive Control Group had the highest levels of these biomarkers.Among the obese populations:Cold Hibiscus Sabdariffa Extracts (250 mg/kg/day) caused the highest concentrations of serum urea, uric acid, and creatinine.Hot Hibiscus Sabdariffa extracts (250 mg/kg/day) resulted in considerably lower levels of biomarkers compared to cold extracts (P<0.05).The study

of the influence of Hibiscus sabdariffa extracts on renal function in obese rats has crucial implications for future therapeutic approaches. The findings, as shown in Table 6, reveal significant alterations in serum urea, serum uric acid, and serum creatinine levels across various experimental groups **(Emelike and Dapper, 2013).** The decline in serum urea levels observed in the negative control group may suggest a protective influence, while the higher levels in the positive control group could signal compromised renal function. Particularly noteworthy is the elevated serum urea in obese groups treated with cold Hibiscus sabdariffa extracts at 250 mg/kg/day, emphasizing the intricate relationship between Hibiscus sabdariffa and renal parameters **(Govindaraj and Rosmarinic, 2015).** Regarding serum uric acid, the heightened levels in the positive control group may indicate renal stress, contrasting with lower levels in the negative control group. Cold Hibiscus sabdariffa extracts show a propensity to increase uric acid levels in obese groups, while hot extracts exhibit a mitigating effect. These findings suggest a nuanced impact of Hibiscus sabdariffa on uric acid metabolism, potentially influencing renal health in the context of obesity **(Alegbe *et al.*, 2019).** The data on serum creatinine levels further contribute to the discussion. The elevated creatinine levels in the positive control group suggest renal impairment, while the negative control group exhibits lower levels. Cold Hibiscus Sabdariffa extracts, particularly at 250 mg/kg/day, induce higher creatinine levels in obese rats, whereas hot extracts show a contrasting effect **(Ritter Ruas *et al.*, 2023).**

Table (6) Effect of Ketogenic Diet (KD) in the presence of Hibiscus Sabdariffa extracts on kidney functions of obese rats

Groups	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
Control –ve	0.81^{c}±.18	31.58 ^{d} ±3.37	3.47^{d} ±0.25
Control +ve	1.13^{a} ±0.05	58.39^{a} ±1.78	7.04 ^{a}±0.08
Obese rats fed on ketogenic diet (KD)	1.01^{b}±0.11	45.95 ^{b}±2.45	5.30 ^{b} ±0.33
Obese rats were fed on KD and treated with 250 mg/kg of cold Hs extract.	0.85^{c} ±0.04	36.37 ^{c}±1.16	4.43^{c}±0.13
Obese rats were fed on KD and treated with 250 mg/kg of hot Hs extract.	0.65^{d} ±0.03	26.93^{e}±1.83	3.34^{d}±0.06
LSD (P≤ 0.05)	0.11	4.08	0.36

Values are shown as mean ± standard deviation. Means in the same column with different superscript letters differ significantly (P < 0.05).

The study indicates that combining the ketogenic diet with Hibiscus sabdariffa significantly enhances the management of obesity. Biochemical analyses demonstrated notable improvements in lipid profiles, liver enzymes, and kidney functions in rats receiving this combination. These findings suggest that this approach holds promise as an effective strategy for obesity management and overall health improvement.Further research with larger and more diverse animal samples is recommended to confirm these findings. Additionally, exploring the underlying biochemical mechanisms of this combination could provide deeper insights. Clinical trials in humans should be conducted to evaluate the efficacy and safety of this dietary approach, and optimal dosages of Hibiscus sabdariffa should be determined to maximize benefits.

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تأثير النظام الغذائي الكيتوني مع مستخلصات نبات الكركديه على التحكم في السمنة لدى الفئران البدينة.

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الملخص العربى

النظام الغذائي الكيتوني هو نظام غذائي عالي الدهون ومنخفض الكربوهيدرات يهدف إلى إحداث حالة من الكيتوزية في الجسم للحصول على الطاقة وتقليل السمنة والدهون في الجسم. تم إجراء الدراسة لفحص آثار الجمع بين النظام الغذائي الكيتوني مع نبات الكركديه للتحكم فى السمنة ودهون الجسم، حيث تم تقسيم خمسة وعشرون فئران ألبينو بيضاء إلى خمس مجموعات، تحتوي كل منها على خمسة فئران، كالتالى. المجموعة ١، مجموعة ضابطة سلبية، تتألف من ذكور الفئران الأصحاء التي تتغذى على نظام غذائي أساسي. المجموعة ٢، المستخدمة كعنصر تحكم إيجابي، تتألف من فئران سمينة تتغذى على نظام غذائي عالي الدهون فقط. المجموعة ٣ تشمل فئران بدينة تم إعطاؤها نظامًا غذائيًا كيتونيا يتكون من ٧٠٪ دهون و ٢٠٪ بروتين و ١٠٪ كربوهيدرات . تم إعطاء المجموعات ٤ و ٥ نظامًا غذائيًا كيتونيًا يتكون من ٧٠٪ دهون و ٢٠٪ بروتين و ١٠٪ كربوهيدرات مع اعطائهم مستخلص الكركديه المائي البارد والساخن (٢٥٠ مجم / كجم / يوم) على التوالى عن طريق الفم، مذاب في الماء المقطر. بعد انتهاء التجربة بمرور ثمانية وعشرين يومًا ، تم تحليل العلامات البيوكيميائية، بما في ذلك مستويات الجلوكوز، وصورة الدهون، وأنزيمات الكبد، ووظائف الكلى. . واكتشفت الدراسة أن الجمع بين النظام الغذائي الكيتوني والكركديه يحسن الصحة بشكل كبير ويعالج السمنة. أدى أعلى تركيز من المستخلص المائي الساخن عن طريق الفم مع النظام الغذائي الكيتوني إلى تحسين مستوى الدهون وإنزيمات الكبد ووظائف الكلى بشكل ملحوظ بين المجموعات التي تعاني من السمنة المفرطة. ويشير هذا البحث إلى أن النظام الغذائي الكيتوني المكمل بالكركديه يمكن أن يقدم علاجًا واعدًا للسمنة.

الكلمات المفتاحية: النظام الغذائي الكيتوني، السمنة، نبات الكركديه ، الجلوكوز، الدهون، الكبد، وظائف الكلى.