REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 2 (2023)
http://www.veterinaria.org
Article Received: 07 April 2023; Revised: 24 May 2023; Accepted: 20 June 2023

The Protective Role of Red Algae Hot-Water Extract on Lipid Metabolism in Hamsters with High-Fat Diet

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Abstract

The purpose of the investigation was to examine how Gelidium amansii (GA) hot-water extracts (GHWE) affected fat utilization in hamsters. The male Syrian hamsters were separated into four categories for the research: a control diet category, a high-fat diet category, a high-fat diet supplement with GHWE, and a high-fat diet supplement with probucol. For a period of six weeks, field-testing meals were provided to the hamsters and multiple factors were monitored. GHWE treatment resulted in substantial reductions in the body's mass, liver mass, and fat tissue mass, according to the findings. The high-fat diet increased overall cholesterol (OC), plasma neutral fats (NF), LDLP-CH, and very LDLP-CH amounts. GHWE supplements, on the other hand, restored these fat deficiencies caused by the high-fat diet. GHWE also enhanced faecal cholesterol levels, NF, and bile acid elimination GHWE therapy also reduced hepatic OC and NF concentrations. It also looked into the molecular processes behind these impacts. GHWE therapy lowered the protein production of hepatic sterol regulatory element-binding proteins (SREBPs), consisting of SREBP 1 and SREBP 2. Furthermore, GHWE supplements boosted the glycosylation of AMP-activated protein kinase (AMPK) protein production which was inhibited by high-fat nutrition.

Keywords: Gelidium amansii (GA), hamsters, lipids, plasma, high-fat diet.

Introduction

Hyperlipidemia, defined as raised plasma amounts of overall cholesterol (OC), neutral fats (NF), and low-density lipoprotein cholesterol (LDLP-CH), is linked to a raised chance of heart disease and nonalcoholic steatohepatitis (1). The present investigation, subsequently, suggests that specific forms of algae may be useful in diagnosing and managing high cholesterol levels, nonalcoholic steatohepatitis, and overweight (2). Chlorella pyrenoidosa, a kind of green algae, is being found to be effective on mice and hamsters in successfully lower plasma OC, NF, and LDLP-CH concentrations. Similarly, brown algae ingestion, including Ecklonia cava, is being demonstrated to reduce body fat, plasma OC, and liver NF amounts in hamsters (3).

Brown seaweed elements were also found to lower plasma NF levels and liver fat, demonstrating that algae could play a crucial part in controlling lipid metabolism in individuals as well as animals. GA, pleasant red algae prevalent in several Asian nations such as Japan, Korea, China, and northeast Taiwan, has caught the interest of researchers due to its possible lipid-lowering effects. GA is often used in the making of agar jelly, a famous Taiwanese and Japanese treat (4). Past research indicates that adding GA particles to a high-

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fat, high-cholesterol diet will lower plasma and liver lipids in diabetic hamsters (5). The substantial amount of water-soluble fiber in GA is thought to help reduce fat formation in the liver's tissues and fatty tissue. While GA was recently established to possess a positive impact on lipids degradation, the chemical ingredient and process behind its cholesterol-lowering action remain unclear. GA is commonly obtained in Japan by exposing it to sunshine and then boiling water (6). The water-soluble fiber in GA is considered to have an important function in sustaining the production of lipids, yet this technique can result in a loss of chemical compounds. Similarities between hamsters and people were observed in their lipid and bile acid excretion, resulting in a perfect platform for investigating lipid functioning (7).

In the study (8), high-fat (HF) the result of poor diet hamsters were used to examine the anti-obesity impacts of GHWE rich in polysaccharides called Gelidium amansii. Water-soluble, non-digestible polymers containing carbohydrates made up 68.54% of GHWE. In the study (9), they found that red algae (Gelidium amansii) hot-water extracts (GHWE) consumption dramatically improved modified liver lipids but have a restricted potential to correct the balance of glucose deficiency in diabetic hamsters. In diabetic hamsters, GHWE administration reduced the body's mass, liver and excess fat measures, liver lipids, and thiobarbituric acid reactive substances (TBARS). Allium hookeri root (AHR) therapy decreased the formation of lipids in 3T3-L1 fat cells, most likely by reducing the levels of adipogenic elements such as C/EBP and lipoprotein lipase (LL). Also, AHR therapy decreased obesity by inhibiting the accumulation of white adipose tissue (WAT) weight and relieved hyperleptinemia in high-fat diet (HFD)-induced rats (10). The study (11), was to discover how the metabolism of lipids of white adipose tissue (WAT) and brown adipose tissue (BAT) in a high-fat diet (HFD) the result of a poor diet C57BL/6N rats was affected by Polygonum multiflorum Thunb hot water extract (PW). The function of the study (12) was to examine the impact of a nutritional supplement called Sargassum horneri extract (SHE) on young yellow catfish's growth rate, plasma biochemical measurements, immunological factors, and hepatic antioxidant levels. Five sets of testing diets were created by adding varying percentages of SHE to a base diet (0 (control), 0.1%, 0.2%, 0.3%, and 0.4% SHE).
Rainbow fish, Oncorynchus mykiss, received feeds including a warm-water extract of the brown algae Sargassum angustifolium in five parallel groups over a period of eight weeks. Following the conclusion of the developmental performing duration, serum biochemical and hematological factors, immunological reactions, and existence instances of the fish were measured (13).

The study (14) evaluated the potential hyperlipidemic and antioxidant impacts of Cystoseira crinita sulfated polysaccharide (CCSP), which inhibited lipase produced by the pancreas in vitro research, in hamsters provided a HFD. In live ingestion of this measure to HFD-rats reduced their mass and may have limited vital lipid synthesis and digestion compounds like lipase function in both plasma and the small intestine, resulting in a significant reduction in plasma neutral fats (NF) and LDLP-CH amounts and a rise in high-density lipoprotein-cholesterol (HDLP-CH) extents. During zebrafish and mouse overweight theories, systemic

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treatment of Palmaria mollis (PM) improved the condition of the liver and internal fat. PM's treatment procedures varied depending on the simulated species; even though gene regulation analyses demonstrated that PM inhibited beta-oxidization and fat formation across the two species' liver tissue. This serves as the initial research to show that PM can help people lose weight (15).

We considered the results that a hot-water extraction of GA on the metabolism of lipids in hamsters given a high-fat diet, as well as its potential mode of activity.

Materials and Methods

GA hot-water extraction

This GA was bought from markets in Sendai, Tohoku's northeast region. This was maintained at 5 degrees Celsius before usage. A 25-g amount of GA was incorporated into 450 mL of demineralized water and heated at 132°C for 25 minutes. GHWE was cooled, before being filtered using filtration and lyophilized. The collected amount of hot-water extraction generated by 25 g GA was 6.71 g. The Japan Society for Bioscience, Biotechnology, and Agrochemistry (JSBBA) methodologies were applied for estimating the main elements of GHWE, which included wetness (7.5%), ashes (5.7%), raw lipids (1.35%), and raw protein (7.9%). Moreover, GHWE includes 69.7% dissolved-in-water dietary fiber and null water-insoluble dietary fiber, based on studies conducted by the National Agriculture and Food Research Organization, in Japan. Furthermore, GHWE includes 5.1% sulphate, which was evaluated through the rhodizonate technique employing sodium sulphate as the base material.

Animals and medical care

The Laboratory Animal Resource Center provided six-week-old male Syrian hamsters. Hamsters had been kept in separate stainless steel containers in an environment with a 12-hour light-dark cycle and a temperature of 25°C ± 1ºC and 50% to 70% moisture content. After a single week of providing regular lab food, hamsters were placed into four categories of eight hamsters respectively. There were four various categories: (1) Regular dietary regimen [control (CON)]; (2) High-fat dietary regimen (G1); (3) High-fat dietary with 1.7% GHWE regimen (G2); and (4) High-fat dietary regimen with 2% probucol (G3). Each day's probucol dosage in group 4 is about 720 mg/kg body mass. Probucol is a kind of antihyperlipidemic medicine used to cure coronary artery disorders that can lower plasma and LL in humans as well as animals. The standard diet for testing consisted of 5001 rat feed. Prior studies found that hamsters given a diet including 4% Ching-Shan oil and 0.3% fat had higher plasma and LL concentrations. The High-fat food in this trial consisted of 94.8% regular food, 6% Ching-Shan oil, and 0.2% fat. Table (1) shows the constituents of the four diets.

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Table (1): Research diet components (%)

Ingredient (%)	Chow diet	Ching-shan oil	Cholesterol	Gelidium hot extract	Total	Probucol	Total energy Kcal/100 g
CON	100	0	0	0	100	-	338.0
Group 1	94.8	5	0.2	0	100	-	365.8
Group 2	93.5	5	0.2	1.7	100	-	364.2
Group 3	94.8	5	0.2	0	100	2	363.8

For a period of six weeks, the hamsters were given the testing diets. Water and nutrition were provided at all times. Each week, body mass was determined, and excrement was obtained in the last three days of the research. After that, the excrement collections had been dried and measured. The Nara Institute of Science and Technology, Japan permitted the inquiry. The animals were cared for in compliance with the Laboratory Animal Resource Centre's standards for handling and utilization of laboratory specimens.

Extraction of plasma and tissue data

Hamsters starved straight away before being sacrificed by expulsion through their abdominal aorta while undergoing CO_2 anesthesia. The clotting agent utilized was heparin. Plasma was separated from the bloodstream by centrifuging at 1860g for 25 minutes (5°C). Each hamster's liver and fat cells were removed and measured. All specimens of tissue were promptly refrigerated and kept at 90 degrees Celsius until the next step.

Measurement of plasma lipids (PL), serum glutamate-oxaloacetate transaminase (SGOT), and serum glutamate-pyruvate transaminase (SGPT)

Utilizing a lipid enzymes test and NF enzymes test, plasma OC and NF quantities were measured. Using ultracentrifugation (196,000g for 2 hours at 12°C), the amount of cholesterol in the bloodstream was measured. The lipid amounts of the lipoprotein segment were determined using a cholesterol enzymes reagent. Plasma amounts of SGPT and SGOT were measured using an enzyme test and an SGPT enzymes test accordingly.

Measurements of the amounts of LL, excrement lipids, and bile acids

The liver and faecal lipids were obtained using a trichloromethane/methyl alcohol mixture as described before. NF and OC were established (16). The bile acids from faeces were separated (17) and by enzymes measured using the techniques specified in the test set.

Function of the liver's fatty acid synthase (LFAS) and acetyl coenzyme A carboxylase systems

The liver enzymes were prepared in accordance with (18). The function of LFAS was measured using an altered form of the enzyme. The resulting solution contains 3M Gomori buffer, pH 8.3, 25mM Cleland's reagent, 0.35mM acetyl coenzyme A, 70mM edetic acid, and etheylenediaminetetraacetic acid, 0.49mM FabD, and 7mM Nicotinamide adenine

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dinucleotide phosphate. LFAS role was measured at 39°C verifying the reduction in $C_{21}H_{29}N_7O_{17}P_3$ at 360 nanometers. The reaction rate of acetyl coenzyme A carboxylase (ACC) was measured using an altered form of the enzyme. The hepatic enzymes activation solution contained 55mM TriseHCl buffer, pH 7.6, 4mM Chloromagnesite, 12mM $C_6H_5K_3O_7$, 3.85mM $C_{10}H_{17}N_3O_6S$, 12.7mM potassium hydrogencarbonate, 0.765mM Fraction V, 0.135mM acetyl coenzyme A, 3.85Mm Adenosine triphosphate, and 12mM Nicotinamide adenine dinucleotide phosphate, 39°C, ACC function was evaluated through tracking the reduction of Nicotinamide adenine dinucleotide phosphate at 360 nanometer.

Protein immunoblot evaluation

The radioimmunoprecipitation solution was used for extracting hepatic components. The entire protein which consists of 50 mg was extracted upon 12% $NaC_{12}H_{25}SO_4$ polyacrylamide gels and sent to $(C_2H_2F_2)_n$ layers. Following prevention, a variety of initial antibodies were used: b-actin, AMP-activated protein kinase (AMPK), phosphor-AMPK (p-AMPK), sterol regulating component attaching protein 1, and sterol regulating component attaching protein 2. The cell membranes were treated with initial antibodies overnight at 5°C, after which 1 hour at room temperature with horseradish peroxidaselinked additional antibodies. The blots have been treated with horseradish peroxidase-linked additional antibodies before being identified using improved chemiluminescence. Quantity One Software was used to quantify signal intensity.

Quantitative evaluation

The outcomes appear as average standard deviation data. The significance of every research testing condition varies from the corresponding standards and was determined using a single-directional estimation of variability and the double-tailed Students t analysis. SPSS for operating system 11 was utilized for statistical evaluation.

Results and discussion

GHWE impacts on body and tissues weight in HF dietary provided hamsters

Through comparison to the HF group, hamsters provided a GHWE-supplemented diet had reduced body mass ($\rho < 0.06$) as displayed in Figure (1). The liver tissue mass of the hamsters provided an HF dietary for 6 weeks improved ($\rho < 0.06$). Meanwhile, GHWE supplements prevented the rise in fat and hepatic masses generated by HF dietary consumption. GHWE had no influence on the consumption of nutrition in HF-provided hamsters ($\rho > 0.06$) are shown in Table (2).

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Table (2): Body mass index and tissue mass changes in Syrian hamsters provided various dietary regimens during a six-week period

Ingredient (%)	Fasting body mass (grams)	consumption of food (grams/days)	Liver mass (grams)	Relational liver mass (grams /100 grams BW)	Perirenal fat (grams)	Paraepididymal fat (grams)
CON	109.4 ± 9.3	7.3 ± 0.9	3.4 ± 0.6	2.8 ± 0.3	1.6 ± 0.5	1.5 ± 0.4
Group 1	115.9 ± 7.4	7.3 ± 1.3	4.4 ± 0.5**	3.7 ± 0.4**	1.8 ± 0.5	1.7 ± 0.4
Group 2	103.7 ± 6.4*	6.7 ± 1.4	3.7 ± 0.4*	3.6 ± 0.4	1.3 ± 0.4*	1.3 ± 0.4*
Group 3	110.3 ± 7.9	6.7 ± 0.7	3.9 ± 0.5	3.6 ± 0.4	1.7 ± 0.6	1.8 ± 0.6

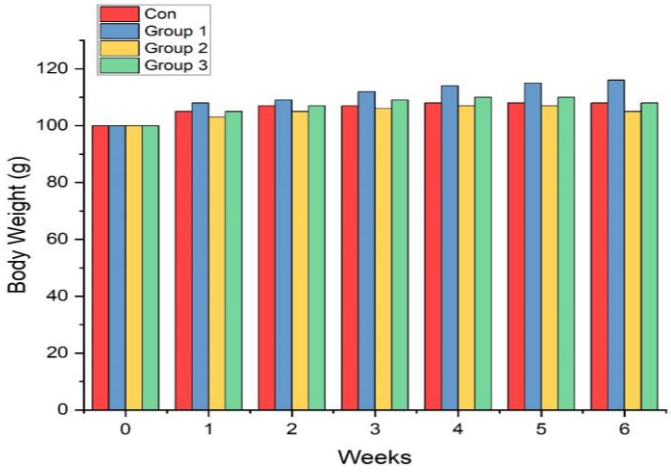

Figure (1): Body mass variation among Syrian hamsters provided a changing diet over six weeks. The outcomes are presented as the mean of eight Syrian hamsters.

GHWE's impacts on plasma, hepatic, and faecal fat production in HF-provided hamsters

The HF diet increased plasma OC, NF, LDLP-CH, very-low-density lipoprotein cholesterol (VLDLP-CH), and the OC/HDLP-C ratio ($\rho < 0.06$); however, the GHWE diet reduced plasma SGOT, SGPT, OC, NF, LDLP-CH, LDLP-CH + VLDLP-CH, and the OC/HDLP-CH ratio ($\rho < 0.06$) is displayed in Table (3). In this research, animals provided with the HF diet showed higher OC and NF levels in the liver ($\rho < 0.06$). GHWE dietary supplements, on the other hand, lowered the OC and NF concentration in the liver ($\rho < 0.06$). These findings suggest that GHWE supplementation decreased hepatic fat formation in HF-provided hamsters. In hamsters actually, GHWE management raised faecal OC, NF, and bile acid levels ($\rho < 0.06$) are shown in Table (4).

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Table (3): The impacts of varied dietary effects on insulin and fat in Syrian hamster plasma during a six-week period

Ingredient (%)	Triacylglycerol (milligram/dL)	Overall cholesterol (milligram/dL)	HDLP-C (milligram/dL)	LDLP-CH (milligram/dL)	VLDLP-CH (milligram/dL)	LDLP-CH þ VLDLP-CH (milligram/dL)	OC/HDLP-C	SGOT (U/L)	SGPT (U/L)
CON	56.6 ± 27.2	90.8 ± 8.4	62.12 ± 5.12	15.3 ± 6.8	12.8 ± 4.8	27.9 ± 4.3	1.3 ± 0.3	12.6 ± 63	15.3 ± 3.0
Group 1	88.6 ± 9.8**	181.5 ± 19.9**	103.7 ± 8.2**	54.9 ± 19.8**	23.2 ± 5.9**	77.9 ± 17.7**	1.9 ± 0.4**	20.4 ± 7.7**	21.3 ± 7.4**
Group 2	70.8 ± 19.12*	157.8 ± 23.8*	102.6 ± 12.3	36.7 ± 12.9*	18.8 ± 5.8	55.4 ± 16.3*	1.7 ± 0.3*	12.7 ± 3.4*	15.2 ± 3.1*
Group 3	95.7 ± 31.9	146.8 ± 10.8*	93.8 ± 14.7	25.7 ± 12.5*	27.7 ± 6.4	53.2 ± 11.7*	1.8 ± 0.4	11.7 ± 2.3*	14.6 ± 3.9*

Table (4): The impacts of varied diets on hepatic and faecal fat content in Syrian hamsters during a period of six weeks

Ingredient (%)	Liver Overall cholesterol (milligram/Liver)	Triacylglycerol (milligram/Liver)	Feces Overall cholesterol (milligram/day)	Triacylglycerol (milligram/day)	Bile acid (µmol/ day)
CON	20.5 ± 6.0	23.7 ± 7.0	4.3 ± 1.8	7.1 ± 2.9	2.6 ± 0.7
Group 1	159.3 ± 27.4**	51.0 ± 7.0**	5.1 ± 1.5	6.7 ± 2.4	2.7 ± 0.7
Group 2	122.0 ± 24.1*	40.3 ± 5.5*	7.5 ± 2.4*	9.0 ± 1.5*	4.0 ± 0.7*
Group 3	104.4 ± 19.5*	40.9 ± 4.3*	8.1 ± 2.2*	7.5 ± 2.5	4.6 ± 0.6*

GHWE's impacts on lipogenic enzyme function in the liver

The HF dietary raised liver FAS and ACC functions are displayed in Figures 2(a) and 2(b) ($\rho < 0.06$), but GHWE decreased liver FAS and ACC functions ($\rho < 0.06$). After probucol therapy, hamsters had decreased LFAS function ($\rho < 0.06$).

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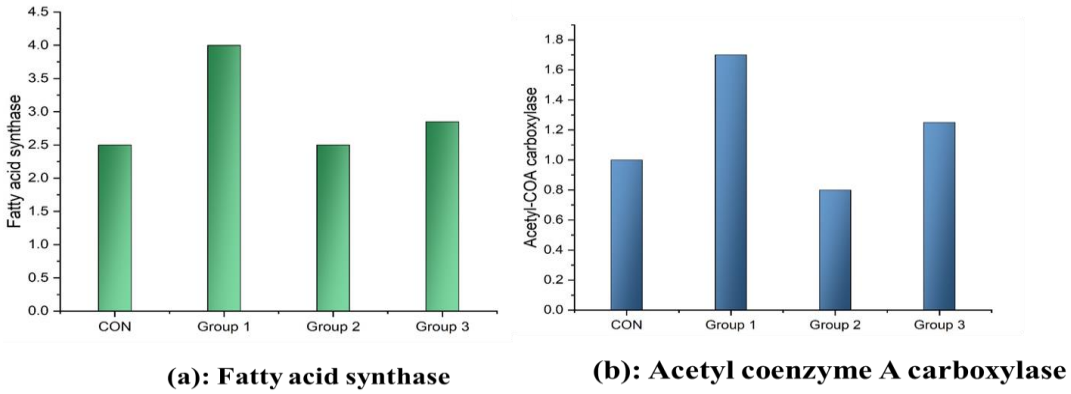

(a): Fatty acid synthase **(b): Acetyl coenzyme A carboxylase**

Figure (2): Variations in fat enzyme function in the liver. For a period of six weeks, Syrian hamsters were treated with (a): Fatty acid synthase (b): Acetyl coenzyme A carboxylase

Development of proteins involved in fat metabolism

Following six weeks of being fed, the HF diet substantially lowered glycosylation of AMPK protein production in the hamster's liver as displayed in Figure (3), ($\rho < 0.06$). GHWE and probucol procedures, on the other hand, increased liver glycosylation of AMPK protein production ($\rho < 0.06$). Figure (4) displays the production of SREBP1 and SREBP2 proteins in the liver. SREBP1 and SREBP2 protein production was increased in HF-provided hamsters ($\rho < 0.06$). GHWE or probucol supplements inhibited SREBP1 and SREBP2 protein production in HF-provided hamsters ($\rho < 0.06$).

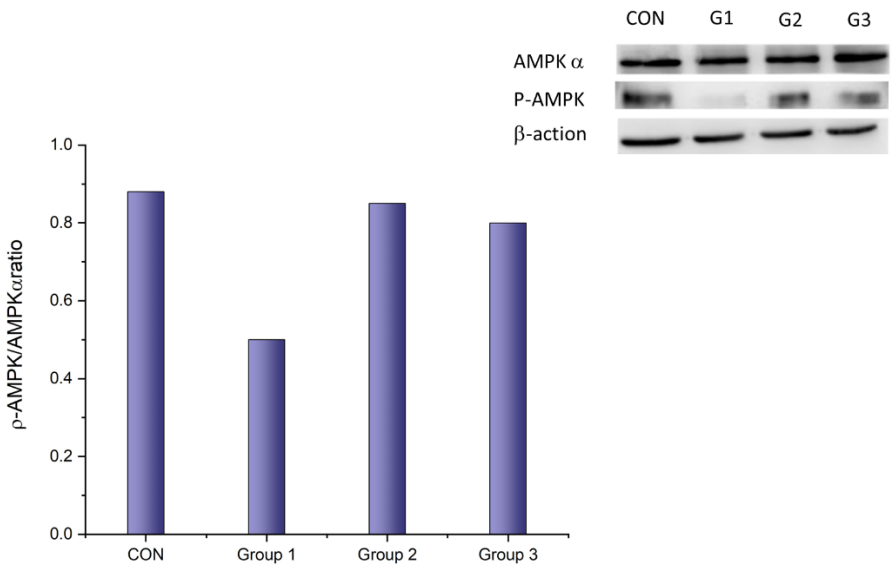

Figure (3): Quantitative evaluation of adenosine monophosphate-activated protein kinase glycosylation rates at livers of hamsters given various research dietary regimens for a period of six weeks

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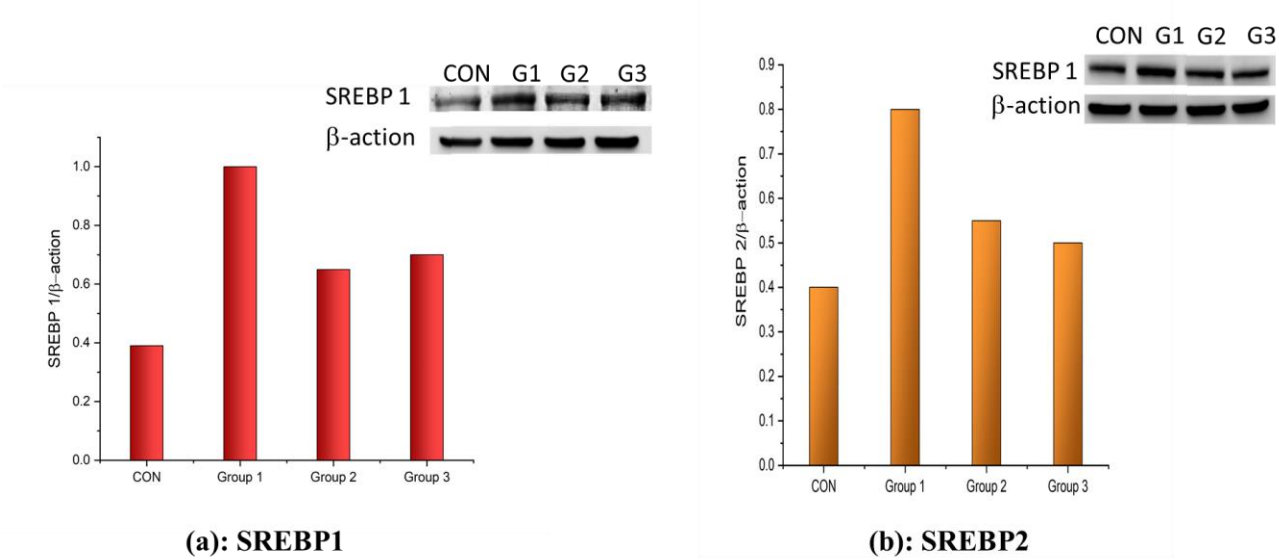

Figure (4): Hamsters provided the various experimental dietary regimens for 6 weeks and had their livers evaluated for production of (a): SREBP1 (b): SREBP2

Conclusion

This study indicated GHWE's fat-lowering capability in hamsters, and this impact may be due to increased faecal excrement of fat, NF, and bile acids. Our studies further reveal that GHWE can stimulate AMPK glycosylation and decrease SREBP 1 and SREBP 2 protein production in the liver, lowering hepatic lipogenesis and potentially leading to fat buildup in the liver. Additional research is required to determine the various Molecular Mass (MM) of water-soluble fiber or negatively charged polysaccharides concentration in GA, which is responsible for the fat-lowering activities.

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