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Research Article
Effects of Chlorophyll on Body Functioning and Blood Glucose Levels

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Abstract

Background and Objective: Blood glucose levels can be affected by the various types of chlorophyll. Experiments have proven that chlorophyll has antioxidant activities that are present in various foods. This study aimed to identify the effects of chlorophyll type on blood glucose and body functioning. **Methodology:** For the study, five different high-chlorophyll products were selected: Mint, broccoli, thyme, grapes and bell peppers. The chlorophyll was extracted from each source. Two types of chlorophyll, chlorophyll a and b, were extracted. The total chlorophyll content was determined. Toxicity tests were conducted using 40 Swiss albino male rats, 6-7 weeks old. The rats were randomly split into 4 groups. The control group was fed *ad libitum* with a Purina® chow diet. The chlorophyll extracts were ground and mixed with the standard pellets so that the feed contained 15% carbohydrate weight replacement with chlorophyll. Student's t-test and the chi-square test were used to assess the significance of the values obtained in both the treated and the control groups during the study. **Results:** Body weights increased after feeding with chlorophyll from all sources except bell peppers. The weight before feeding was 334.10±26.5 g, after feeding, it was 318.7±26.96 g, which is interpreted as a low difference. The mean glucose level was monitored 0, 1, 2 and 3 h after the intake of chlorophyll. A diet rich in chlorophyll led to a slight decrease in the number of white blood cells, haematocrit, haemoglobin and an increase in red blood cells compared with control. The results of the treatment did not show any significant changes in the levels of total cholesterol, LDL, HDL, triglycerides, glucose, urea and creatinine among the experimental and control groups. Moreover, there was significant difference ($p<0.05$) in the weights of the animals' organs among the groups. **Conclusion:** It is concluded that chlorophyll extracts from mint, broccoli, thyme and bell pepper are likely to have important implications regarding blood sugar. Bell pepper extracts and juice has benefits in body weight and further studies are warranted.

Key words: Body functioning, chlorophyll, extraction, juices, body weight

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Glucose is an instant source of energy but when consumed in high concentration, it leads to major problems. The blood glucose level is the amount of sugar or glucose present in the blood stream. The body naturally regulates glucose levels as a part the body's metabolic processes. It is a primary energy mechanism for cells and the blood lipid profile. Bloodglucose is transported from the intestines/liver to the cells via the bloodstream. The absorption of glucose is promoted in the presence of insulin, a hormone produced in the pancreas. If blood glucose levels are not balanced, both high and low blood glucose leads to the malfunctioning of body organs[1]. Fruit and vegetables contain phytochemicals that may be prevent diabetes by regulating glucosidase and lipase activities, reducing the postprandial glycaemic level, providing anti-inflammatory activity and improving pancreatic function and synergistic action with hypoglycaemic drugs[2].

Fruit and vegetables have been reported to contain antioxidant compounds that are valuable in human health, such as carotenoids, vitamin C, vitamin E and phenolic and thiol (SH) compounds[2-4]. The consumption of fruit and vegetables is part of the Arabic cultural heritage and they play important roles in the customs, traditions and food culture of the Saudi household. Mint (*Menthapulegium*), broccoli (*Brassica oleracea*), thyme (*Thymus vulgaris*) and *Gongronema latfolium* are green leafy vegetables that contain a fair amount of chlorophyll. The consumption of a diet rich in fruits and vegetables that are rich in chlorophyll is associated with lower incidences of oxidation-linked diseases, such as diabetes and cardiovascular disease[2-4].

Increased consumption of phytochemicals that have antioxidant activity can suppress the development of diabetes[5]. In contrast to other phytochemical compounds, such as phenolic components, chlorophyll is present in large quantities in plants (±1% dry weight); therefore, it has the potential to be a functional food[6]. Chlorophyll is a component that may help to prevent disease. Moreover, the chlorophylls are the most abundant natural pigment and is considered a plant-derived antioxidant[7]. They are called dihydroporphyrins because they contain four pyrrole rings coordinated with magnesium metal. Along with the porphyrin ring, they also contain a long hydrophobic sidechain derived from an alcohol called phytol ($C_{20}H_{39}OH$), which is responsible for the hydrophobicity of the whole molecule. Generally, higher-level plants contain two types of chlorophyll: a and b. These chlorophyll types differ in terms of the R group, which may be an aldehyde group in chlorophyll b or a methyl group in chlorophyll a[7].

The role of chlorophyll as a hypoglycemic agent occurs through the inhibition of free radicals. Chlorophyll is an antioxidant chain breaker that donates its electrons to free radicals and forms complexes with peroxyl radicals to generate a stable product[8]. Ferruzzi *et al.*[9] reported that dietary chlorophyll derivatives prevalent in both fresh and processed foods and dietary supplements have antioxidant and antimutagenic activities. There are many imported foods based on chlorophyll and the highest chlorophyll content occurs in the green fruits and vegetables mint, broccoli, thyme, grapes and bell peppers. The aim of this study was to evaluate the chlorophyll content of mint, broccoli, thyme, grapes and bell peppers which are commonly consumed in Saudi.

MATERIALS AND METHODS

Preparation of products

Extraction of chlorophyll: First, five products (mint, broccoli, thyme, grapes and bell peppers) with a high phenol content were collected from local vendors. They were referred to as FP 1 (mint), FP 2 (broccoli), FP 3 (thyme), FP4 (grapes) and FP 5 (bell peppers), respectively. Then, whole chlorophyll was extracted and the chlorophyll a and b component contents were determined according to AOAC[10] methods. Table 1 shows the total chlorophyll a and b components per 100 g.

Preparation of samples: Fresh grapes juice was prepared by homogenizing the fruit right before the start of the experiments (1 mL of juice contained approximately 2 g of fresh grapes). The mint, broccoli, thyme and bell pepper leaves were washed, weighed (100 g L^{-1}) and triturated with water in a blender for 7 min. The juice was filtered and frozen in a flask placed in a refrigerator. Each flask was thawed daily at ambient temperature two hours prior to administration.

Animal testing methods: The study was approved by the Department of Nutrition and Food Science, Princess Nourah Bint Abdulrahman University, Riyadh, Saudiin September 2011. Using chronic modes, toxicity studies were conducted with 40 male Swiss albino rats, aged 6-7 weeks and with different weights. The animals were bred and raised at the Animal House, Central Laboratory for Drug and Food Analysis, Ministry of Health, Riyadh, Saudi Arabia. The animals were handled in accordance with the guidelines for selecting doses for long-term treatment and minimal toxicity[11]. The rats were randomly split into 4 groups (10 rats per group). The environment was maintained under standard conditions with a humidity of 50%, a temperature of 22±2°C and light conditions of 12 h each for dark and light.

All the animals were given free access to water. The control group was fed *ad libitum* with a Purina® chow diet purchased from the Arabian Agricultural Services Company (Arasco), Riyadh, Saudi Arabia. The feeds given to the experimental groups were prepared according to the American Institute of Nutrition (AIN) guidelines[12]. Dose selection was based on the regulatory guidelines requiring minimal toxicity during long-term treatment. As body weight and water intake increased over time, the daily dose was adjusted to approximate the initial human therapeutic dose specified by the manufacturer. The extracts were ground and mixed with standard pellets so that 15% of the feed's carbohydrate weight was replaced with the experimental products. The feed was stored at 5°C until it was used. Table 2 summarizes the control and experimental diets. The juices were administered orally at a dose of 0.29 g kg^{-1} once a day. The dose administered to the animals was based on 100 g L^{-1}, which corresponds to a daily intake of 200 mL of juice by an adult man weighing 70.0 kg (this intake was based on population consultation). The diets were labelled FP 1 mint, FP 2 broccoli, FP 3 thyme, FP 4 grapes and FP 5 bell peppers.

Parameters assessed

Toxicity: It was measured as suggested by the WHO Scientific Group[13] and Wilson *et al.*[11].

Weight change: Body weights were recorded before and after the experimental period to examine the impact of the different drugs on body weight. Furthermore, at the end of the treatment, the weights of the vital organs (heart, lungs, kidneys, spleen, liver and testes) were weighed and reported per 100 g b. wt., to facilitate comparisons between the normal and control animals.

Haematological and biochemical analysis: These were assessed before and after the experimental period. The haematological profiles included White Blood Cell count (WBC), Red Blood Cell count (RBC), haemoglobin (Hb), haematocrit (HCT) and the Mean Corpuscular Volume (MCV). Blood was also collected and the serum was separated and stored at -20°C for later biochemical analysis of liver and kidney function and lipid profile. Specifically, alanine aminotransferase (ALT), aspartate aminotransferase (AST)[14], glucose[15], urea[16], creatinine[17], cholesterol; triglycerides[18], High Density Lipoprotein (HDL) cholesterol[19] and Low Density Lipoprotein (LDL) cholesterol[20] were measured. The parameters were analysed with enzymatic colorimetry using test combination reagents (Boehringer Mannheim GmbH, Diagnostica, Germany). Measurements were made using a spectrophotometer (Introspect II, LKB).

Oral Glucose Tolerance Test (OGTT): Oral glucose tolerance was measured after fasting rats were given a glucose solution

Table 1: Total chlorophyll a and b components per 100 g

Sources	Test-ref	Chlorophyll a (ppm)	Chlorophyll b (ppm)	Total
FP 1	AOAC 972.04	549.00	201.78	750.78
FB 2	AOAC 972.04	75.47	32.00	107.47
FP 3	AOAC 972.04	393.10	585.88	978.98
FP 4	AOAC 9772.04	3.79	1.45	5.24
FP 5	AOAC 972.04	31.66	9.69	41.35

FP 1: Mint, FP 2: Broccoli, FP 3: Thyme, FP 4: Grapes, FP 5: Bell pepper

Table 2: Constituents of control and experimental diets in g/100 g

	Quantity of nutrients per 100 g					
Chemical components	Control	FP 1	FP 2	FP 3	FP 4	FP 5
Corn-starch	46.57	39.58	39.58	39.58	39.58	39.58
Casein	14.00	14.00	14.00	14.00	14.00	14.00
Dextrinised corn-starch	15.50	15.50	15.50	15.50	15.50	15.50
Sucrose	10.00	10.00	10.00	10.00	10.00	10.00
Soybean oil	4.00	4.00	4.00	4.00	4.00	4.00
Fiber	5.00	5.00	5.00	5.00	5.00	5.00
Mineral mix	3.50	3.50	3.50	3.50	3.50	3.50
Vitamin mix	1.00	1.00	1.00	1.00	1.00	1.00
L-cystine	0.18	0.18	0.18	0.18	0.18	0.18
Choline bitartrate	0.25	0.25	0.25	0.25	0.25	0.25
Extract (15% carbohydrate)	-	6.99	6.99	6.99	6.99	6.99
Total	100.00	100.00	100.00	100.00	100.00	100.00

FP 1: Mint, FP 2: Broccoli, FP 3: Thyme, FP 4: Grapes, FP 5: Bell pepper

(1.75 g kg^{-1} of weight of the rats). Blood glucose was measured after 1, 2 and 3 h and the test was repeated once a week. The average of the readings was taken.

Statistical analysis: Statistical analysis was performed using SPSS v.16 (Statistical Package for Social Sciences, version 16, SPSS, Inc., Chicago, USA)[21]. Student's t-test and the chi-square test were used to assess the significance of the values obtained in both the treated and the control groups during the study. p values of <0.05 were considered statistically significant[22].

RESULTS AND DISCUSSION

Effect of chlorophyll extraction and juices on body weight: Table 3 shows that the rats' weightsincreased gradually after consuming chlorophyll in the form of mint, broccoli, thyme, grapes but not as a result of bell pepper extract consumption. The pre-treatment value was 334.10±26.50 g, whereas the post-treatment value was 318.70±26.96 g. Both the difference and the standard deviation were low. The consumption of chlorophyll extracts led to growth and the increase in weight reached as much as 1.27 g day^{-1} [8].

Additionally, changes in the body weights of male rats were also observed with the administration of chlorophyll in the form of juices made from mint, broccoli, thyme, grapes and bell peppers. Again, the intake of bell pepper juice was associated with a decrease inthe body weight of the male rats. Dias[23] demonstrated that bell peppers contain substances that lead to increases in body's heat production and oxygen consumption for approximately 20 min after eating. Under such circumstances, the body burns extra calories, which helps with weight loss.

Effect of chlorophyll extracts and juices on the Oral Glucose Tolerance Test (OGTT): The mean glucose level of the male rats was monitored at 0, 1, 2 and 3 h after the intake of chlorophyll in the form of mint, broccoli, thyme, grape and bell pepper extracts and juices. The resultsindicated that treatment with chlorophyll extract led to decreased blood glucose after 3 h (Fig. 1). The highest decrease occurred in the rats that were fed thyme extract. Nissa *et al.*[8] demonstrated that higher doses of chlorophyll extract led to blood glucose levels. The mechanism of chlorophyll as ahypoglycemic agent occurs through its inhibition of free radicals. Chlorophyll is an antioxidant chain breaker that donates its electrons to free radicals and forms complexes with peroxy radicals to generate

Table 3: Effect of chlorophyll extracts and juices on weight

	Body weight (g)	
Treatment groups	Pre-treatment**	Post-treatment**
Control	339.24±19.39	391.89±24.41
Mint extract	339.24±19.39	394.80±10.52
Broccoli flower extract	336.90±21.50	395.80±27.44
Bell pepper extract	334.10±26.50	318.70±26.96*
Thyme extract	338.80±27.92	396.80±32.64
Grape extract	333.40±11.40	389.40±9.00
Control 2	339.24±19.39	391.89±24.41
Mint juice	338.06±19.90	395.06±10.79
Broccoli flower juice	336.18±22.06	396.09±28.15
Bell pepper juice	339.93±27.19	326.99±27.66*
Thyme juice	334.75±28.65	388.82±33.49
Grape juice	339.21±11.70	394.40±9.23

*Compare between the experimental group and control, *p<0.05 (student t-test), **Mean±Standard Deviation

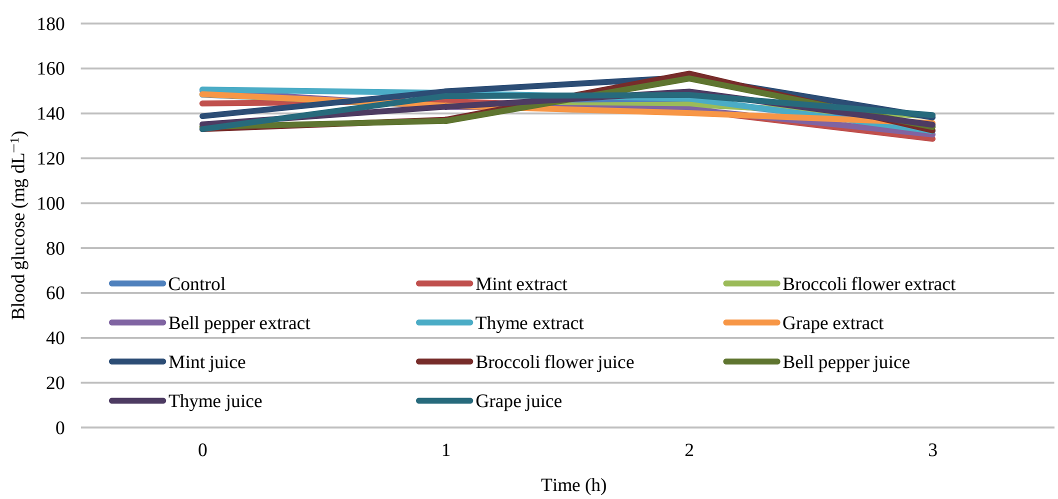

Fig. 1: Effect of chlorophyll extracts and juices on oral glucose tolerance test results

a stable product[7]. In addition to chlorophyll, chloroplasts also contain exogenous superoxide dismutase[24] and ascorbic acid[25], which also have antioxidant capacity. Paradoxically, grape juice led to an increase in blood glucose, while grape extract had the opposite effect, possibly because of the high sugar content of grapes compared with the other fruits and vegetables used.

Effects of chlorophyll extracts and juices on haematological parameters: Table 4 shows the effects of the chlorophyll products on the haematological profile of experimental animals. Itshows that the group fed with chlorophyll experienced a slight decrease in the number of white blood cells and an increase in red blood cells compared with controls. The decreases in white blood cells may have occurred because chlorophyll playsananti-bacterial role[26]. The results indicated that the treatment did not lead to any significant changes.

Data analysis indicated that there was no significant difference between the treatment groups and the control group in terms of lipid profile and blood glucose levels. Figure 2 shows that there were no significant differences in the levels of total cholesterol, LDL, HDL, triglyceride, glucose, urea and creatinine among the experimental and control groups. These results agree with the study of Aminian *et al.*[27], who showed that unripe grape juice consumption had no measurable effect on serum HDL-C levels in healthy individuals. They also stated that grape juice had no effect on TG, total cholesterol and LDL-C[27]. Some studies found that daily consumption of grapes or grape juice reduced body weight, blood pressure and lipids compared with the control[28].

Table 4: Impact of chlorophyll extracts and juices on the haematological test results

	Haematology tests				
Treatment groups	WBC** ($\times 10^{1}$ L^{-1})	RBC** ($\times 10^{12}$ L^{-1})	Hb** (g dL^{-1})	MCV** (fl)	HCT** (%)
Control	8.98±0.11	8.08±0.13*	16.78±0.23	51.32±0.92	46.58±0.66
Mint extract	8.67±0.10	8.74±0.16	15.04±0.27	53.07±0.95	42.66±0.76
Broccoli flower extract	8.92±0.37*	8.70±0.16	16.32±0.26	49.56±0.89	41.42±0.74
Bell pepper extract	8.51±0.37*	8.57±0.15	16.53±0.26	48.53±0.87	42.45±0.76
Thyme extract	8.23±0.20*	8.18±0.15	15.84±0.27	49.36±0.88	42.45±0.76
Grape extract	8.11±0.13*	8.13±0.15	16.32±0.26	51.52±0.92	40.39±0.72
Mint juice	8.78±0.10	8.91±0.16	16.34±0.27	54.12±0.97	43.51±0.78
Broccoli flower juice	8.33±0.38	8.87±0.16	16.61±0.26	50.55±0.91	42.25±0.76
Bell pepper juice	8.91±0.37	8.74±0.16	15.82±0.27	49.50±0.89	43.30±0.78
Thyme juice	8.46±0.21	8.34±0.15	15.13±0.27	50.34±0.90	43.30±0.78
Grape juice	8.25±0.13	8.29±0.15	16.61±0.26	52.55±0.94	41.20±0.74

WBC: White blood cell, RBC: Red blood cell, Hb: Haemoglobin, MCV: Mean corpuscular, HCT: Haematocrit, *Compare between the experimental group and control, *$p<0.05$ (student t-test), **Mean±Standard Deviation

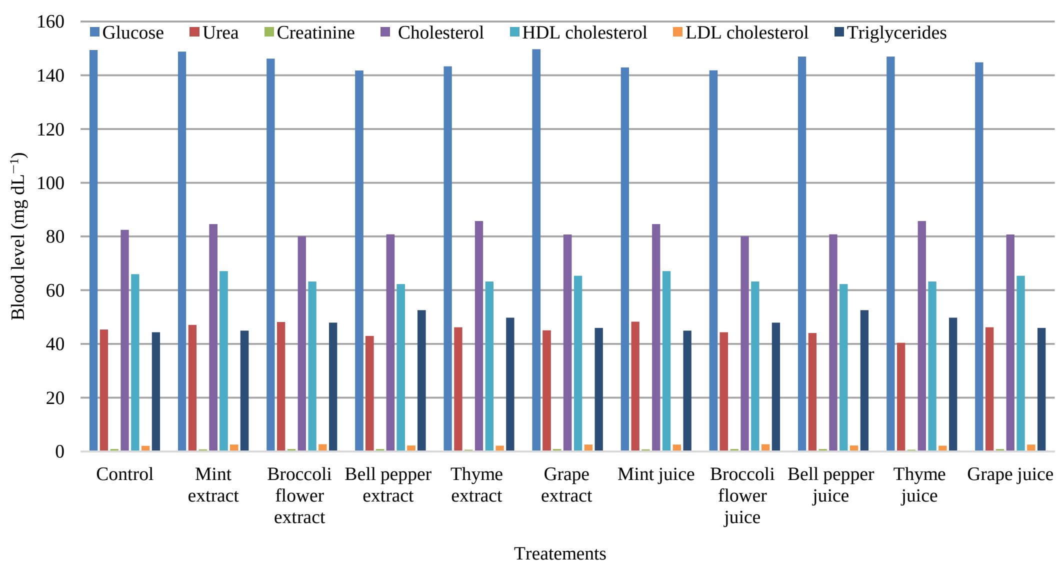

Fig. 2: Effect of chlorophyll extracts and juices on lipids profile and glucose level

Table 5: Effect of chlorophyll extracts and juices on organ weights

	Organ weight changes (g/100 g b.wt.)					
Treatment groups	Heart**	Lungs**	Liver**	Spleen**	Testes**	Kidneys**
Control	1.7±0.03	1.59±0.04	10.99±0.27	1.02±0.03	1.88±0.05	1.29±0.03
Mint extract	1.33±0.03	1.79±0.04	10.25±0.28	1.13±0.03	1.88±0.05	1.32±0.03
Broccoli flower extract	1.28±0.03	1.73±0.04	10.96±0.32	1.06±0.03	1.83±0.05	1.31±0.03
Bell pepper extract	1.58±0.03	1.67±0.04	10.58±0.26	1.04±0.03	1.81±0.05	1.10±0.03
Thyme extract	1. 5±0.03	1.63±0.04	10.98±0.27	1.04±0.03	1.92±0.05	1.05±0.03
Grape extract	1.59±0.028	1.465±0.036	10.577±0.263	0.983±0.024	1.846±0.05	1.015±0.025
Mint juice	1.129±0.033	1.789±0.044	11.247±0.280	1.130±0.028	1.883±0.05	1.318±0.033
Broccoli flower juice	1.38±0.03	1.73±0.04	11.96±0.32	1.06±0.03	1.83±0.05	1.31±0.03
Bell pepper juice	1.18±0.03	1.67±0.04	10.58±0.26	1.04±0.03	1.81±0.05	1.10±0.03
Thyme juice	1.015±0.025	1.632±0.041	10.975±0.27	1.036±0.03	1.915±0.05	1.046±0.026
Grape juice	1.12±0.03	1.47±0.04	10.58±0.26	0.98±0.02	1.95±0.05	1.02±0.025

*Compare between the experimental group and control, *p<0.05 (student t-test), **Mean±Standard Deviation

There was also no significant difference in the weights of the animals' organs among the groups (Table 5).

CONCLUSION

Consumption of chlorophyll of mint, broccoli, thyme, grapes led to increase of weight while chlorophyll of bell pepper demonstrated decreased the weight. Based on the findings in this study, a key observation was that chlorophyll was the key factor related to increase in weight and decreased blood glucose. It can be concluded that include chlorophyll rich food and its extraction in diet positively influence on health.

SIGNIFICANCE STATEMENT

This study discovers the potential of chlorophyll in the form of mint, broccoli, thyme, grapes as a source of phytochemicals that can be beneficial for humannutrition. This study will help the researcher to uncover the critical areas of food applications that can use chlorophyll chemical constituents. Thus, new information about chlorophyll in the form of macro- and micronutrients can be obtained and used in a practical way.

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