Effect of different levels of guava leaves on the biochemical parameters of obese rats

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Abstract

The present study was designed to evaluate the effect of different levels of guava leaves on body weight, serum cholesterol, total triglyceride, liver functions and kidney functions. Forty male rats, weighing between 150±5 g. were divided into 5 groups. The first group fed on high fat diet as control group, the other groups fed on a high fat diet with 5, 10, 15 and 20 g guava leaves /day. The obtained results indicated that a significant increase in feed intake, body weight and organs weight for obese control group while adding the guava leaves led to significant decrease in body weight and organs weight especially at the level 15 and 20 %. It could be observed that total cholesterol, triglycerides, kidney functions and liver functions were decreased by increasing the level of guava leaves intake. The values of hemoglobin and hematocrit when compared with the control group were increased by increasing the levels of guava leaves except the high dose (20%). So the study recommended to use a natural source of fiber and antioxidant as V.C. The level of 15%was able to improve the health status of obese rats .

Keywords: -Serum cholesterol- liver functions- kidney functions- weight gain.

Introduction

Industrialization has led to many modifications in the lifestyle of the world's populations, giving rise to increase the indices of several diseases, including chronic degenerative diseases such as insulin resistance, diabetes mellitus, dyslipidemia, metabolic syndrome and cardiovascular diseases, reducing the quality of life and increasing costs on hospitalizations, medications and other public health interventions **(Sharma and Chandola, 2011).** Studies have demonstrated that the consumption of fruits, vegetables and seeds can be helpful to prevent the risk factors of many diseases due to the bioactive compounds. Many plants have been used for the purpose of reducing risk factors associated with the occurrence of chronic disorders and for many other purposes [**Bamosa *et al*., 2015**]. Psidium guajava L. is a small medicinal tree that is native to South America. It is popularly known as guava (family Myrtaceae) and has been used traditionally as a medicinal plant throughout the world for a number of ailments. There are two most common varieties of guava: the red (P. guajava var. pomifera) and the white (P. guajava var. pyrifera) (**Kaneria and Chanda , 2016)**. All parts of this tree, including fruits, leaves, bark, and roots, have been used for treating stomachache and diarrhea in many countries. Leaves, pulp and seeds are used to treat respiratory and gastrointestinal disorders, and as an antispasmodic, anti-inflammatory, as a cough sedative, anti-diarrheic, in the management of hypertension, obesity and in the control of diabetes mellitus. It also possesses anticancer properties (**Ru *et al.*, 2017**). Guavas leaves are being rich in vitamins A and C and especially dietary fiber. The amount of vitamin C is (100-104 mg per 100 gm serving) and also has good levels of the dietary minerals, potassium, magnesium, and generally a broad, low-calorie profile of essential nutrients . The high cost of pharmaceutical medications conduces to the search for alternative medicines to treat many ailments. In view of this, studies are necessary to confirm the effects of medicinal plants **(Hung *et al.,* 2017**) .Therefore, this research is conducted to study the effect of different levels of guava leaves on serum cholesterol, total triglyceride, liver functions, kidney functions, and hemoglobin of obese rats.

Materials and methods

Materials

. Guava leaves (***Psidium guajava***) was purchased as crude dried material from Local Company for Medicinal Plant and Spices, Cairo, Egypt. Male albino rats of Sprague Dawley strain (40 rats) their weight was between (150±5 g) were obtained from Institute of Ophthalmology Medical Analysis Dep., Giza, Egypt.

Experimental design

Rats were housed individually in well crated cages under hygienic conditions and fed for 7 days on high fat diet with 10% saturated animal fat adlibitum in the biological laboratory of Research Institute of Ophthalmology Medical Analysis Dep., Giza, Egypt. The standard diet consisted of casein (12%), corn oil (10%), 10% saturated animal fat , vitamin mixture (1%), salt mixture (4%), fiber (cellulose) (5%), choline chloride (0.2%) and starch up to 100g according to (**Hegested , 1941, Campbell, 1963 and Reeves *et al.,* 1993).** Rats were divided into five groups (8 rats each), the first group was fed on high fat diet guava leaves at the levels of 5, 10, 15 and 20%.

Methods

Chemical analysis

Chemical composition

Moisture, ash, crude protein (Kieldhl, TN × 6.25), fat (Soxhlet using hexane) and vitamin C contents of guava leaves were determined according to methods described by the **A.O.A.C. (2005).** Carbohydrates of guava leaves were estimated by difference as follows:

% Carbohydrate = 100- (% moisture+ % protein + % fat + % ash **+ %Fiber).**

Biological study

Biological evaluation

Biological evaluation of the different diets was carried out by determination of body weight gain (BWG), feed intake and organs ⁄body weight ratio according to **Chapman *et al.,* (1959).**

Blood sampling

At the end of experimental rats were starved for 12 hr, and then scarified under ether anesthesia, blood samples were collected from the aortic vein into clean dry centrifuge tubes and were stored at room temperature for 15 min., put into a refrigerator for 2 hr, then centrifuged for 10 minutes at 3000 rmp to separate serum. Serum was carefully aspirated and transferred into dry clean wasser-man tubes using a Pasteur pipette and then kept frozen at (-20ºC) till analysis.

Biochemical analysis

Some blood samples were taken in heparinized micro tubes to estimate both hemoglobin and hematocrit according to **Mc Inory (1954).** Red blood cells (RBC-s) were calculated as follows: RBC-s= Hb×0.35. Total cholesterol was determined by the method of **NIHP (1987),** while triglycerides were determined according to **Jacobs and Van Denmark (1960)**. Serum creatinine was determined according to the method described by **Henry, (1974).** Serum urea was determined according to the method described by **Patton and Crouch (1977)**. Determination of ALT was carried out according to the method of **Yound (1975) and Henry (1974),** while AST according to the method of **Tietz (1976).**

Statistical analysis

Standard error and ANOVA test followed by the student *t*-test for significant difference. Statistical significant difference was defined as $P < 0.05$ **(Snedecor and Cochran, 1976).**

Results and discussion

1- Chemical composition of dried guava leaves.

Data in table (1) showed the content of moisture which was 14.57 % and crud protein, fat, ash, fiber and carbohydrates in guava leaves (on dry weight) which were 2.6, 1.1, 3.5, 11.43, and 81.37 %respectively . It could be noticed that guava leaves rich in carbohydrates and fiber and the obtained results matched with **Rincón *et al.,* (2001)** who found that the guava leaves content from dietary fiber was between 8-13 % and the total carbohydrates was between 75- 83% according to the kind of guava and source of it.

Table (1): Chemical composition of dried guava leaves.

Constituents	Dried Guava leaves
Crud protein	2.6
Fat	1.1
Ash	3.5
Fiber	11.43
Carbohydrate	81.37

2- Effect of different levels of guava leaves on feed intake, body weight gain and organs to body weight ratio.

Data in table (2) showed high level of guava leaves resulted in a high decreasing of body weight gain compared with the control group while , it caused increasing in feed intake except the level 20% when compared to control group .It may due to the high amount of leaves which cuased a bitter taste **(Wang and Chen, 2008).** So, the lowest values of feed intake and body weight gain of rats were in group fed on 20% of guava leaves.

Weight of all organs liver, kidney and spleen were changed as a result of different levels of guava leaves in the diet of rats when compared to the control group. The statically analysis showed a significant at the level 20% when compared to the other groups and the control group. It can be observed that the values of liver, kidney and spleen weight /body weight were in normal results when compared with the control group except its values at level 20% were lower than the other groups. Organ's relative weight such as liver, heart, kidney, lung, spleen and testes (**Bergamaschi *et al.,* 2011**) were not affected by the guava leaves supplementation. They were not significantly different as compared to control group . Organ weight measurement is important to access general toxicity because any change in organ weight is a sensitive indicator of toxicity. Liver is the target organ because most toxicants enter the body via the gastrointestinal tract, and after absorption, the toxicants are carried by the hepatic portal vein to the liver. In theory, organ weight will be affected by the suppression of body weight as described by **Gosmann *et al.* (2012)**. In this study, the guava leaves supplementation did not result in any significant changes in the relative weights of organs of obese rats as compared to control groups. Administration of guava leaves for obesed rats showed no toxic signs such as nose bleeding, vomiting, fur loss, diarrhea and death throughout the observation period. The administration of the highest dose used in the experiment did not show any toxicity effects and could be considered safe (**Zhao *et al.,* 2015).**

Effect of different levels of guava leaves on feed intake, body weight gain and organs to body weight ratio of obese rats

.Values with same letters indicate no significant difference $p < 0.05$

3- Effect of different levels of guava leaves on lipid profile of obese rats

From this study, it found that daily administration of guava puree leaves showed positive results in significantly reducing total cholesterol and LDL-C levels (Table 3) after four weeks which is similar to a study by **Gosmann *et al.* (2012)**. The group that received higher level showed a 34.47% reduction in total cholesterol (TC) levels followed by the level 15% (23.30%) and 10% (22.33%) respectively as compared to control group. The triglyceride (TG) levels of the treated groups showed a decrease after four weeks of treatment. The highest reduction in TG levels was in the level 20% with 43.59% followed by 15% (34.19%) and 10% (32.48%). High density lipoprotein-cholesterol (HDL-C) levels showed an increase in 10% (13.33%), 15% (13.33%) and 20%(18.75%) as compared to control group. The low density lipoprotein-cholesterol (LDL-C) levels showed a reduction in value, for the level 20% (69.70%), 15% (39.40%) and 10% (37.12%) respectively as compared to control group. The higher reduction in TC levels in treated groups may be due to the increased excretion of bile acid. Guava leaves is reported to have high crude fibre and mineral content, especially potassium, sodium, magnesium, phosphorus, zinc and iron (**Yamashiro *et al.*, 2010**). **Zhao *et al.*, (2015**) reported that moderate feeding of guava leaves caused changes in dietary fatty acids and carbohydrates. Some researchers claimed that a diet rich in vegetables and fruits can prevent atherosclerosis. However, rats fed with a high level of guava leaves 15% showed no any inhibitory effects and this may be probably due to the high concentration of cholesterol in liver and plasma because of cholesterol feeding (**Bergamaschi *et al.*, 2011**). Obesity that is related to hypercholesterolemia and hypertriglyceridemia is a major risk factor for the development of cardiovascular disease. Oxidatively damaged LDLs are taken up by macrophages, which accumulate in the endothelial wall as lipid-laden foam cell in the initial phases of atherosclerotic fatty streak lesions. Therefore, a reduction in circulating TGs, TC and LDLs is primary in prevention of vascular disease. In addition, prevention of LDL oxidation by dietary antioxidants could delay the development of atherosclerosis (**Citation *et a*l., 2012). In** the present study, feeding rats with high level of guava leaves treated obese rats.

Table (3): Effect of different levels of guava leaves on lipid profile of obese rats(mmol/L)

Parameters	Control group	Levels of guava leaves %			
		5	10	15	20
TC	2.06 [a] ± 0.26	1.95 [b] ± 0.39	1.60 [b] ± 0.37	1.58 [b] ± 0.32	1.35 [c] ± 0.22
TG	1.17 [a] ± 1.13	1.04 [a] ± 0.40	0.79 [b] ± 0.18	0.77 [b] ± 0.18	0.66 [b] ± 0.16
HDL-C	0.30 [b] ± 0.10	0.36 [b] ± 0.13	0.43 [a] ± 0.10	0.45 [a] ± 0.13	0.48 [a] ± 0.07
LDL-C	1.32 [a] ± 0.18	0.97 [a] ± 0.39	0.83 [b] ± 0.22	0.80 [b] ± 0.27	0.40 [c] ± 0.08

Values with same letters indicate no significant difference p <0.05

Effect of different levels of guava leaves on some kidney and liver functions of obese rats

Table 4 shows significant changes (p<0.05) in urea concentrations in group (5) fed on 20% guava leaves (36.25 ± 1.56 mg/dl) followed by 15% (43.97± 1.62 mg/dl) and 10% (50.01 ± 4.55 mg/dl) respectively, as compared to control group (62.46 ± 2.23mg/dl). Creatinine concentrations did not show any significant differences between treated group with 5% and control group. Kidney is the second organ most frequently affected by any compounds **(Marshall, 2000).** Therefore, renal functions can be assessed by measuring the concentrations of creatinine and urea in plasma **(Moshi et al., 2001).** Previous reports showed that some herbal preparations used for a long period are associated with kidney injury. Plasma urea and creatinine concentrations are often used as an index of renal glomerular function and will be increased in renal injuries . Urea is synthesised in the liver, primarily as a by-product of the deamination of amino acids. Creatinine, a by-product from muscle mass, will affect its concentration in blood (**Bergamaschi *et al.*, 2011**).

Table (3) shows the activities of serum enzyme (AST and ALT) concentrations. There is no significant differences in AST parameter between groups 5% and 10%. There were significant differences (p<0.05) in ALT and AST among treated groups with 10, 15 and 20% as compared to control group . Alanine aminotransferase (ALT) was significantly lower in 20% (28.25 ±5.9U/L), followed by 15% (37.4 ±4.3 U/L) and 10% (47.1±4.7 U/L) respectively, as compared to CN

(55.6 ±2.1 U/L). Liver function test is crucial because liver is the central organ in detoxification of compounds. In general, enzymes provide an excellent marker of tissue damage. Organ or tissue damage causes the release of increased amounts of many enzymes into the blood stream (**Rajamanickam *et al.*, 2015**). **Rai *et al.***,(**2016**) reported that the activities of most enzymes normally detectable in blood remain constant in healthy and normal person . This shows that the synthesis of protein in the guava leaves induced obese rat's liver is not influenced by the supplementation. Similar results were also obtained in the studies onguava leaves. A healthy liver is so crucial for protein metabolism since liver disease is frequently associated with alterations in proteins and disturbances of protein metabolism . Total protein and albumin concentrations will be decreased by inadequate synthesis due to liver disease(**Moshi et al., 2001**).

Table (3): Effect of different levels of guava leaves on some kidney and liver functions of obese rats

Parameters	Control group	Levels of guava leaves %			
		5	10	15	20
Creatanin mg/dl	1.58 ±0.91 a	1.46 ±0.72 a	1.30 ±0.67 b	1.21 ±0.43 b	0.94 ±0.11 c
Urea mg/dl	62.46 ±2.23 a	57.23 ±3.74 a	50.01 ±4.55 b	43.97 ±1.62 b	36.25 ±1.56 c
AST U/L	145.2 ±3.14 a	132.1 b ±3.6	129.3 ±5.21 b	120 ±3.13 c	108.2 ±4.22 d
ALT U/L	55.6 a ±2.1	52.8 a ±1.6	47.1 b ±4.7	37.4 c ±4.3	28.25 d ±5.9

Values with same letters indicate no significant difference $p < 0.05$

4- Effect of different levels of guava leaves on hemoglobin, red blood cell and hematocrit.

When the percentage of red blood cells and hemoglobin concentration fall, a physician suspects that it is a case of iron deficiency. Physicians also use these two measures to assess iron status, along with the amount of iron and iron containing proteins in the blood stream. In cases of sever deficiency hemoglobin falls so, low that the amount of oxygen carried in the blood stream is decreased. Such a person has anemia defined as a decreased oxygen carrying capacity of the blood (**Gordon, 2000**).Our results revealed that Hb%, RBCs and

HCT values are higher in control group than the groups fed on diet containing 5, 15, 10 and 20 % of guava leaves. The lowest group is found in group which received diet containing 5% guava leaves. The difference between this group and the control group was significant at p<0.05.The obvious increase of in Hb%, RBCs and HCT values in blood groups of 20% artificial of guava leaves was due to improve the absorption of iron by ascorbic acid content of guava leaves. These results are in agreement of with **Marshall, (2000)**.Anemia is considered as one of the most common index of malnutrition over the world and is caused by iron deficiency store (IDS) or iron-deficiency erythropoietin (IDE) based on the screening criteria for iron-deficiency anemia (**Gordon, 2000**). Data in table 4 indicated that iron was significantly (P<0.05) increased as affected by increased sources of ascorbic acid when guava intake level (15 ang 20%).

Table (4): Effect of different levels of guava leaves on hemoglobin, red blood cell and hematocrit.

Parameters	Control group	Levels of guava leaves %			
		5	10	15	20
Hemoglubin %	13.38±3.07 a	9.5±2.37 c	10.1±1.74 c	10.8±3.82 b	11.5±2.08 b
Red blood cell %	4.65±1.12 a	3.29±0.12 b	3.45±1.14 b	3.65±1.78 b	3.98±1.12 a
Hematocrit (p.v %)	39.89±1.12 a	29.9±1.14 c	32.6±4.96 b	33.9±2.77 b	35.9±1.44 b

Values with same letters indicate no significant difference p <0.05

Conclusion

The present study, concluded that the using of guava leaves at the level 15 % (lead to increase in feed intake and decresed body weight, organs weight while, total cholesterol, and triglycerides . Kdney functions and liver functions were enhanced. In addition with increase in hemoglobin, hematocrit and red blood cells . It is suggested that the maximum level of guava leaves 15% to avoid liver and kidney toxicity.

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تأثير المستويات المختلفة من اوراق الجوافة على المقاييس البيوكيميائية للفئران المصابة بالسمنة

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صممت هذه الدراسة الحالية لتقييم تأثير المستويات المختلفة من اوراق الجوافة على وزن الجسم ، كوليستيرول السيرم ، الجليسريدات الكلية ، وظائف الكبد، وظائف الكلية. أجريت التجربة على اربعون فأر من الذكور أوزانهم بين ١٥٠±٥ جم قسمت إلى ٥ مجموعات . المجموعة الأولى غذيت على الوجبة الضابطة وهى تمثل المجموعة الضابطة والمجموعات الأخرى غذيت على الوجبات الضابطة مضاف إليها ٥، ١٠، ١٥ و ٢٠% من ورق الجوافة) .وقد أوضحت النتائج وجود زيادة معنوية في المأخوذ من الطعام ، ووزن الجسم ووزن الأعضاء للمجموعة الضابطة المصابة بالسمنة. بينما عند اضافة اوراق الجوافة وخاصة عند المستويات ١٥ و ٢٠ % ادت الى انخفاض معنويا لوزن الجسم ، كوليستيرول السيرم ، الجليسريدات الكلية ، وظائف الكبد، وظائف الكلية . مستويات الهيموجلوبين والهيموتوكرت عند المقارنة بالمجموعة الضابطة زادت بزيادة نسبة الاضافة لاوراق الجوافة ما عدا المجموعة التى تحتوي على ٢٠% من الاوراق . لذلك توصى الدراسة باستخدام اوراق الجوافة كمصدر طبيعي للالياف والمواد المضادة للاكسدة مثل فيتامين سي . المستوى ١٥% كان قادر على تحسين الحالة الصحية للفئران المصابة بالسمنة .

الكلمات الكاشفة : –كوليستيرول السيرم– وظائف الكبد– وظائف الكلية– زيادة الوزن