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Evaluation of Antihyperglycaemic Activity of Citrus Peels Powders Fortified Biscuits in Albino Induced Diabetic Rats

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Abstract Citrus peels, waste by-products of citrus juices factories are reckoned valuable healthful functional food. This investigation evaluated antihyperglycaemic activity of citrus peels powders fortified biscuits in induced diabetic rats. 10% fortified biscuits with peels powders of four studied citrus Groups, namely: Diabetic fed basal diet (DFBD) + 10% fortified biscuits (FB) with Baladi orange peels powder (PP); DFBD + 10% FB with Abo-Sora orange (PP); DFBD + 10% FB with Tangerine (PP); and DFBD + 10% FB with Baladi lemon (PP) were assessed. Two extra groups; Group fed Basel diet (BD), and Group fed BD + 10% wheat biscuits were used as control. The effect of six studied groups on body weight (BW) and serum glucose content of albino rats was assessed. Data revealed that the four studied citrus groups reduced BW of rats, but the highest decrease was recorded in group DFBD + 10% FB with Baladi orange (PP), suggesting that FB with 10% citrus (PP) could be recommended for caloric reduced diets for obese, over-weight and diabetic persons. Data showed that DFBD + 10% FB with Baladi lemon (PP) recorded noticeable decrement in glucose serum of experimental rats recommending it for diets of diabetic persons.

Keywords Citrus Peels , 10% Fortified Biscuits, Antihyperglycaemic Activity , Diabetic Rats , Body Weight , Serum Glucose , Baladi orange , Abo-Sora Orange , Tangerine , Baladi lemon

1. Introduction

Citrus peels are the skins of fruits such as lemons, oranges, tangerines, limes or grapefruits. Recording to Medical Herbs & Spices, According tow Fraley (2010) citrus peels have been known for their high levels of vitamin c and its associated health benefits[1].

Citrus genus is the most important fruit tree in the world and lemon is the third most important citrus species. Several studies highlighted citrus fruits as an important health-promoting fruit rich in phenolic compounds as well as vitamins, minerals, dietary fiber, essential oils and carotenoids (Gonzalez-Molina *et al*.[2]; Manthey & Grohmann[3]; Nogato *et al*.[4]; Cohen *et al*.[5]; Thiel[6]; Chedea *et al*.[7]; Gross[8]; Rincon *et al*.[9]; Hui[10]; Marks[11]; Marty[12]; Huang & Ho[13]; Xu *et al*.[14]; and Starling[15]).

Moreover citrus peels have a strong commercial value for the fresh products market and food industry. Besides citrus peels productive networks generate high amounts of wastes and by-products that constitute an important source of bioactive compounds with potential for animal feed, manufactured foods, and health care. The citrus fruit residues, remaining after juice extraction, represent approximately half of the wet mass of the whole fruit, including (flavedo and albedo) and almost one-fourth of the whole fruit mass.

Citrus fruit peel is the outer skin of the covering of the fruits. The peel is being recognized as one of the essential component of our diet as it contains many vital nutrients and non-nutrient compounds which play important role in well being.

Citrus peel is low in calories, sugar, and fats; and is free from cholesterol[16,17].

Furthermore, the antihyperglycaemic activity of methanol extract of citrus peel in streptozotocin-induced diabetic rats was reported by Nagappa *et al*.[18], Murali *et al*.[19]; Li[20]; Haldar *et al*.[21]; Oyelola *et al*.[22]; Panda *et al*.[23]; which quite agrees with Horner[24]; Wolf[25]; and Kundusen *et al*.[26]. Citrus peel had been recommended in traditional herbal medicine as the source of diabetic remedy for diabetes[27,28,29].

According to Fraley[1] citrus peel extract containing polymethoxylated flavones (PMFs) may help prevent

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diabetes. Marks[11] reported that the pericarp, the white membrane of orange peel (the source of pectin which is associated with lower cholesterol, improves insulin resistance. Wolf[25] stated that the pectin of the orange peel (as a natural fiber) can decrease the rise in blood sugar that may occur after a meal, which quite agree with Horner[24] and Amerman[30]. Michael[31] reported that grapefruit contains naringenin, an antioxidant that increases insulin sensitivity by accelerating the breakdown of fat in the liver.

The present investigation was designed to evaluate antihyperglycaemic activity of citrus peel fortified biscuits in albino induced diabetic rats as well as their effect on their body weight.

2. Materials and Methods

2.1. Materials

10kg wheat flour 72% extraction hard red winter were obtained from El-Haram Milling Company, Faesal, Giza in January 2012. Sugar powder, corn oil, sodium chloride, ammonium bicarbonate, and rose oil were purchased from Assiut local market in January 2012. 20 kg of each of the four studied citrus fruits were procured from Assiut University Horticulture Farm namely: Baladi orange, Abo-Sora orange, Tangerine and Baladi lemon in January 2012. The citrus fruits were peeled and the obtained peels were sun dried to complete dryness on wood trays. The dried peels were milled by hammer mill to produce citrus peels powders. The citrus peel powder was kept in glass containers at 4°C in the refrigerator till the analysis.

2.2. Technological Process

2.2.1. Biscuit Formula and Ingredients

Control biscuit dough was prepared according to the formula presented in table (1),[32]. The supplemented biscuits with citrus peels powders were prepared using the same formula except for replacing the wheat flour with 10% of each of the four studied citrus peels powders.

Table 1. Biscuit formula*

Ingredients	Amount (g.)
Wheat flour (72% extraction)	100.00
Powdered sugar	25.00
Corn oil	15.00
Sodium chloride	1.00
Ammonium bicarbonate	1.00
Rose oil	0.01 (one drop)
Water	20.00

* Saba (1997).

2.2.2. Dough Preparation

Powdered sugar and corn oil were creamed in Braun Mixer with a flat beater for 2 minutes at 6 rpm. Water containing sodium chloride, ammonium bicarbonate and rose oil was added to the cream and mixed for 5 minutes at 125 rpm to obtain a homogenous cream. Thereafter flour was added slowly to the above cream and was mixed for 2 minutes at 60 rpm to obtain biscuit dough[32].

2.2.3. Preparation of Biscuit

The dough was sheeted to a thickness of about 3 mm using Atlas Brand rolling machine. The sheeted dough was cut into round shape using a 45 mm diameter cutter and baked on an aluminium tray in an electric oven at 180°C for 6 minutes. The biscuit was cooled for 30 minutes, packed in polyethylene bags under desiccation[33,34].

2.2.4. Preparation of Different Blends of Biscuits

Blends of biscuits were prepared using wheat flour 72% extraction rate as control or those which substituted with 10% of Tangerine peel powder, 10% Abo-Sora orange peel powder, 10% Baladi orange peel powder, and 10% Baladi lemon peel powder.

3. Methods

3.1. Biological Experiment

3.1.1. Experimental Animals

Sixty adult male white albino rats (Sprague dawley strain) weighing between 100 and 120 grams were obtained from the animal house of the Faculty of Medicine, Assiut University. The animals were housed as groups in wire cages under the normal laboratory conditions and were fed on basal diets. The rats were fed for a week as adaptation period. Body weight was weighed weekly and at the end of the experimental feeding period.

3.1.2. Basal Diet Constituents

The basal diet used is outlined in Tables (2), (3), and (4).

Table 2. Constituents of the basal diet for 100 gm diet*

Item	%
Corn starch	67.8
Casein	12.5
Corn oil	10.0
Vitamin mixture	1.0
Salt mixture	3.5
Cellulose	5.0
Choline chloride	0.2
Total	100.0%

*El-Sayed[35] and Ilwy[36].

Table 3. Constituents of vitamins mixtures used in the basal diet*

Vitamins mixtures	
Item	Amount (gm)
Vitamin A palmitate 500.000 IU/gm	0.80
Vitamin D_3 100.00 IU/gm	1.00
Vitamin E acetate 500 IU/gm	10.00
Menadione sodium bisulfite 62.,5% mendadione	0.08
Biotin 1.0%	2.00
Cyano cobalaming 0.01%	1.00
Folic acid	0.20
Nicotinic acid	3.00
Calcium pantothenate	1.60
Pyridoxine HCl	0.70
Riboflavin	0.60
Thamin-HCl	0.60
Sucrose	978.42
Total	1000.00

* Anon[37]

Table 4. Constituents of the salt mixture used in the basal diet*

Salt mixture	
Item	Amount (gm)
Calcium phosphate, dibase 29.5% Ca, 22.8% P	500.00
Magnesium oxide 60.3% ug	24.00
Potassium citrate, 1 H_2O, 36.2% K	220.00
Potassium sulfate 44.9% K, 18.4% S	52.00
Sodium chloride 39.3% Na, 60.7% Cl	74.00
Chromium potassium sulfate 12.0 H_2O, 10.4% Cr	0.55
Cupric carbonate 57.5 Cu	0.30
Potassium iodate 59.3% I	0.01
Ferric citrate 21.2% Fe	6.00
Manganous carbonate 47.8% Mn	3.50
Sodium selenite 45.7% Se	0.01
Zinc carbonate 52.1 Zn	1.60
Sucrose	118.03
Total	1000.00

* Anon[37]

3.1.3. Experimental Design

The rats were randomly divided into 6 groups of 10 rats each. Each rat was marked on the tail to differentiate between animals. Daily administration were continued for (4) weeks. Group (1) was fed with the basal diet and was used as control. Group (2) was injected intramuscularly with alloxan monohydrate ($C_4H_2N_2O_4.H_2O$)[39] in single dose of 170 mg/kg body weight (Diabetic) and fed with the basal diet + 10% fortified biscuits with Baladi orange peels powder. Group (3) was injected as group (2) and fed with the basal diet + 10% fortified biscuits with Abo-Sura orange peels powder. Group (4) was injected as group (2) and fed the basal diet + 10% fortified biscuits with Tangerine peels powder. Group (5) was injected as Group (2) and fed the basal diet + 10% fortified biscuits with Baladi lemon peels powder. Group (6) was injected as group (2) and fed the basal diet + 10% wheat flour 72% extraction biscuits.

3.1.4. Blood Sampling

At the end of each experiment, rats were fasted overnight and anesthetized. Blood samples were collected from all animals from the retro-orbital plexus of each group into clean, dry and labeled tube. The tubes contained heparin (10.0 IU/ml) as anticoagulant. Blood was centrifuged (3500 rpm for 15 min) to separate plasma which was tightly kept in sealed aliquot tubes at -20°C until biochemical assay of serum glucose was carried out.

3.1.5. Determination of Serum Glucose

Serum glucose level was analyzed by colorimetric procedures kits developed by Diamond Diagnostics Kits Cairo, Egypt using 550 nm according to Tietz[38]. Serum glucose was estimated by the enzymatic colorimetric treatment with GDD-DAP method as follows:

To a 10 serum, 10 ml of the enzymatic solution at 37°C for 10 minutes was added and the obtained color was read at 550 nm using Beckman DU-6μ aspects photometer. Standard glucose and blank samples were read and the value of glucose was obtained using the following equation by Vender[38].

$$\text{Serum glucose (mg/100 ml)} = \frac{100 \text{ x } \Delta A \text{ (sample)}}{\Delta A \text{ (standard)}}$$

where 100 = The concentration of the standard solution and ΔA = The absorbance.

3.2. Statistical Analysis

The data were analyzed statistically using SAS computing procedure. The least significant difference and correlation coefficient were calculated for all means using the procedure of[39].

4. Results and Discussion

4.1. Body Weight Gain

The results given in Table (5) revealed that the body weight gain was negative in all the six studied groups expect that in the group 6 which recorded positive body weight gain for the experimental rats (induced hyperglycaemia).

The data showed that the mean values both decrements and increment in the body weight gain in the six studied groups after 28 day feeding trial were 129.40↓, 120.42↓, 131.00↓, 130.98↓, 136.98↓, and 142.85↑. However, the lease decrease among the six studied groups was recorded in group 2 (Diabetic fed basal diet + 10% fortified biscuits with Baladi orange peels powder), Which recorded a mean of 120.42 g by the end of the feeding time of the experiment. While, the only increment in the body weight gain was recorded in group 6 (Diabetic fed basal diet + 10% wheat flour 72% extraction biscuits which recorded a mean of 142.85 g. by the end of the feeding time of experiment.

Similar findings were reported by Kundusen *et al*.[26], who reported that the final body weights were decreased in *citrus limetta* fruit peel in streptozotocin-induced diabetic rats. Wolf[25] stated that the citrus peel (orange peel) is a very good source of pectin, which can help diminish an

overactive appetite, which leads to unwanted weight gain. Horner[24] reported that grapefruit peels powder achieved weight loss, which is in good agreement with our data. On the basis of such findings in the present investigation fortified biscuits with 10% citrus peels powders could be recommended for caloric reduced diets for obese, over-weight persons and diabetic persons.

Table 5. Effect of the six studied groups on the body weight (g) of the experimental rats (induced hyperglycaemia)

Time of observation	Control (BD)	Diabetic + 10% FBBOPP	Diabetic + 10% FBASOPP	Diabetic + 10% FBTPP	Diabetic + 10% FBBLPP	Diabetic + 10% WFB
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Initial weight (g)	146.67	143.33	147.50	146.67	140.00	131.00
End of 1st week (g) % change	135.00↓ 5.52%↓	123.75↓ 13.60%↓	132.50↓ 10.17%↓	143.75↓ 1.99%↓	138.75↓ 0.88%↓	138.75↑ 5.91%↑
End of 2nd week (g) % change	128.33↓ 12.50%	115.00↓ 19.77%↓	128.33↓ 13.00%↓	123.75↓ 15.62%↓	137.33↓ 1.66%↓	139.00↑ 6.11%↑
End of 3rd week (g) % change	120.00↓ 18.18%↓	110.00↓ 23.25%↓	125.00↓ 15.25↓	118.25↓ 19.38%↓	135.33↓ 3.33%↓	152.50↑ 16.41%↑
End of 4th week (g) % change	117.00↓ 20.23%↓	105.00↓ 26.74%↓	121.67↓ 17.51%↓	112.50↓ 23.30%↓	132.67↓ 5.24%↓	153.00↑ 16.70%↑
Mean (g) % change	129.40↓ 11.77%↓	120.42↓ 15.98%↓	131.00↓ 11.86%↓	130.98↓ 10.69%↓	136.82↓ 2.27%↓	142.85↑ 9.05%↑

Group 1 = Fed basal diet (control)
Group 2 = Diabetic fed basal diet + 10% fortified biscuits with Baladi orange peels powder
Group 3 = Diabetic fed basal diet + 10% fortified biscuits with Abo-Sora orange peels powder
Group 4 = Diabetic fed basal diet + 10% fortified biscuits with Tangerine orange peels powder
Group 5 = Diabetic fed basal diet + 10% fortified biscuits with Baladi lemon orange peels powder
Group 6 = Diabetic fed basal diet + 10% wheat flour 72% extraction biscuits

4.2. Antihyperglycaemic Activity

The results given in Table (6) and Figure (1) revealed that the blood serum glucose showed slight increment in the six studied groups, except that group 5 (Diabetic + 10% FBB (PP) and group 6 (Diabetic + 10% WFB) noticeable decrement (28.75%↑ and 21.91%↑), respectively. The present data given in Table (6) and Figure (1) on blood serum glucose in the serum of the experimental animals agree with Kundusen *et al.*[26], who reported that in STZ-induced diabetic rats, *citrus limetta* fruit peel demonstrated a potential antihyperglycaemic effect which may be attributed to its antioxidant property. The fruits peel of citrus contains flavonoids hesperidin and naringin, which both are proven to be potent hypoglycaemic agents as reported by Manthay and Grohmann[3], Ali and Abdel-Kader[28], Jung *et al.*[29], Nogata *et al.*[4] and Perez *et al.*[40].

Table 6. Effect of the six treatments on serum glucose content of the experimental rats, with induced hyperglycaemia

Time of observation	Control (BD)	Diabetic + 10% FBBOPP	Diabetic + 10% FBASOPP	Diabetic + 10% FBTPP	Diabetic + 10% FBBLPP	Diabetic + 10% WFB
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
End of 1st week	42.540	42.540	42.540	42.540	42.540	42.540
End of 2nd week (g) % change	45.751 7.54%↑	48.891 14.92%↑	48.014 12.86%↑	46.859 10.15%↑	35.011 17.69%↓	28.129 33.87%↓
End of 3rd week (g) % change	50.623 19.00%↑	46.813 14.74%↑	44.157 3.80%↑	38.591 9.28%↓	26.374 38.00%↓	26.628 37.40%↓
End of 4th week (g) % change	38.221 10.15%↓	47.229 11.02%↑	47.529 11.72%↑	42.933 1.15%↑	17.321 59.28%↓	36.790 13.52%↓
Mean (g) % change	44.284 4.09%↑	46.368 8.99%↑	45.560 7.09%↑	42.731 0.45%↑	30.311 28.75%↓	33.522 21.19%↓

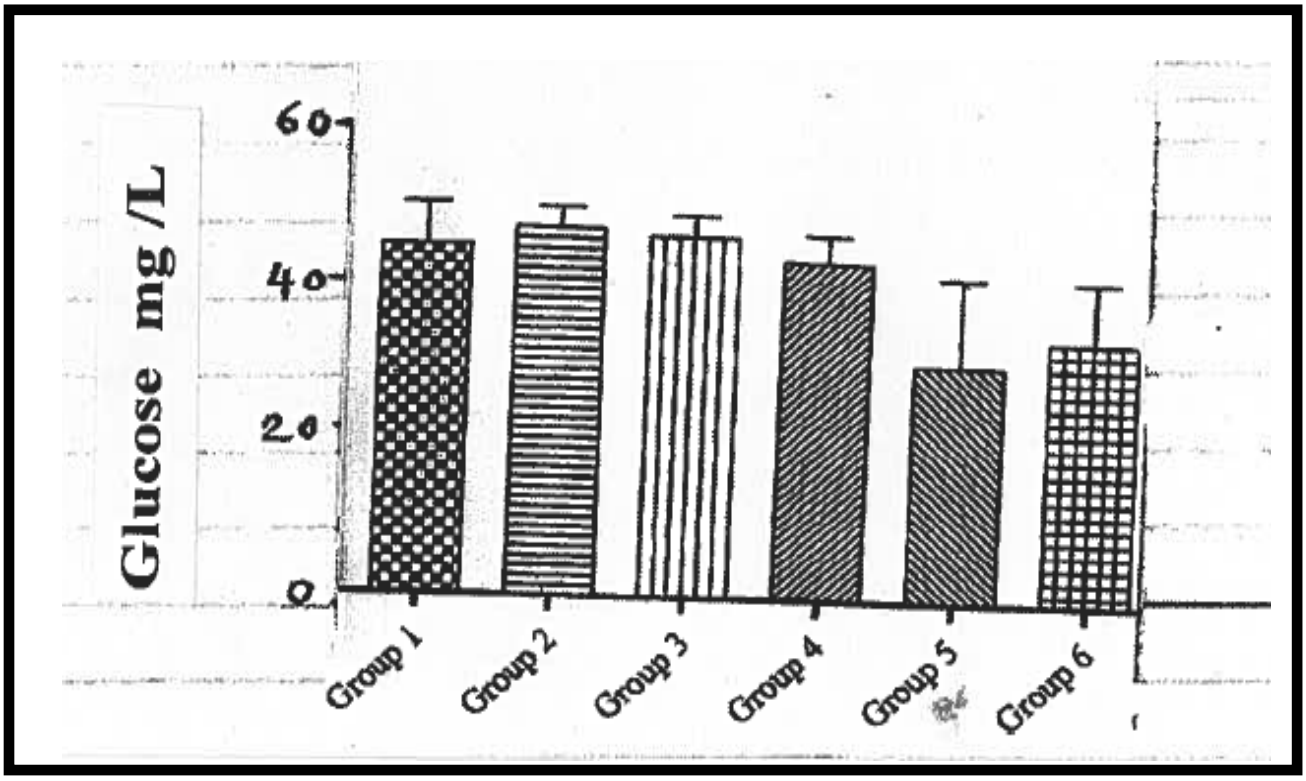

Figure 1. Mean ± SD of serum glucose content of the experimental rats (induced hyperglycaemia) in the six studied groups

Table 7. Least significant difference test of serum glucose content of the experimental rats (induced hyperglycaemia) in the six studied groups

Group	Mean	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
		44.284	46.368	45.560	42.731	30.311	33.522
Group 1	44.284	0					
Group 2	46.368	2.084	0				
Group 3	45.560	1.276	0.808	0			
Group 4	42.741	1.553	3.637	2.829	0		
Group 5	30.311	13.973***	16.057***	15.249**	12.420**	0	
Group 6	33.522	10.762*	12.846**	12.038**	9.209*	3.211	0

* = P < 0.05 ** = P < 0.01 *** = P < 0.001
$LSD_{0.05}$ = 8.915 $LSD_{0.01}$ = 12.005 $LSD_{0.001}$ = 15.916

Furthermore, citrus peel extracts contain polymethoxylated flavones (PMFs) which may help prevent diabetis as well as may improve insulin sensitivity as reported by Fujioka[42], Li[20], Akiyama *et al*. (2010), Fraley[1] and Marks[11].

Table (7) outlined least significant difference test of glucose content of the experimental rats induced hyperglycaemia and correlation coefficient among the six studied groups. The data showed that the correlation coefficient between Group (1) (BD, control) and groups (2), (3) and (4) were not significant. Meanwhile, the correlation coefficient between Group (1) and Group (5) and Group (6) were high significant, and significant; respectively. This explains the high hyperglycaemic activity of Group (5) (Diabetic + 10% FBBLPP), which suggests that 10% fortified biscuits with Baladi lemon peels powder could be recommended for the diet regimen of diabetic persons.

Such finding is in good agreement with Harborn[41] and Kundusen *et al*.[26].

In conclusion, the four studied wheat biscuits fortified with 10% citrus peels powders, namely: Group 2 (Diabetic fed basal diet + 10% fortified biscuits with Baladi orange peels powder), Group 3 (Diabetic fed basal diet + 10% fortified biscuits with Abo-Sora orange peels powder, Group 4 (Diabetic fed basal diet + 10% fortified biscuits with Tangerine peel powder, and Group 5 (Diabetic fed basal diet + 10% fortified biscuits with Baladi lemon peel powder reduced the body weight of the experimental rats by the end of the feeding time of the experiment. However, Group 2 (Diabetic fed basal diet + 10% fortified biscuits with Baladi orange peel powder recorded the least decrement in the body weight of the experimental animals. Therefore, fortified biscuits with 10% citrus peels powders could be recommended for caloric reduced diets for obese, over-weight persons and diabetic persons.

On the other hand, Group 5 (Diabetic fed basal diet + 10% fortified biscuits with Baladi lemon peel powder recorded noticeable decrement in serum glucose of the experimental

rats by the end of the feeding time of the experiment. Therefore, it could be recommended for the diet regimen of diabetic persons.

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