

The Role of Natural Antioxidants and Snacks on the Weanling Rats Health

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Abstract: The effect of processed snacks, commercial snacks and commercial snacks + orange juice on the lipid profile, haematogenic characteristics, liver and kidney functions, glucose and malonaldehyde (MDA) in weanling rats were evaluated. Weight gain and histological examination of liver and kidney tissues were also evaluated. The results showed that there were no significant ($P>0.05$) in hemoglobin, haematocrit, red blood cell, glucose and creatinine between control and other snacks groups. Rats fed commercial snacks resulted in a significant ($P\leq 0.05$) increase in total cholesterol, triglyceride, low density lipoprotein (LDL), very low lipoprotein (VLDL), alanine amino transferase (ALT), aspartate amino transferase (AST), urea, creatinine and malonaldehyde compared to rats fed basal diet. However, supplementation commercial snacks diet with orange juice as a source of antioxidant resulted in significant ($P\leq 0.05$) decrease in the previous parameters and improves the liver and kidney tissues as compared with commercial snacks diet.

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1. Introduction:

We live in society where it is easy and convenient to eat unhealthy food and difficult to eat healthy food (1). Any food that has poor nutritional value, lack in micronutrients such as vitamins, minerals, amino acids, fibers and high content level of calories is considered unhealthy and may be called a junk food such as some snacks and breakfast cereals. Snacks and breakfast cereals are essentially produced from starchy substances such as corn, rice, wheat (2). Snacks food is easy to carry, purchase and consume. Junk food is given a very attractive appearance by adding food ingredients. The major role of these ingredients is to give structure, texture, mouth feel, bulk, and many other characteristics desired for specific finished products (3 and 4). Fast food consumption has been associated with higher total energy intake and higher intake of fat, saturated fat, carbohydrates, sugar, and carbonated soft drinks and lower intake of micronutrients and fruit and vegetables (5-11). In addition to the high consumption of fast foods contributes to passive overeating, weight gain and obesity in humans (12,13,14). Obesity is a major risk factor for many chronic conditions, including hypertension, cardiovascular disease, type-2 diabetes, as well as certain types of cancer (15). An increased consumption of fruit and vegetables has been associated with beneficial effects on the risk of disease (16,17) The beneficial effect could be related to minor components, especially flavonoids, which are proposed to exert their action by inhibiting LDL oxidation(18) and vitamins C and E and beta-

carotene, which are thought to act mainly as antioxidants (19). Citrus juices, especially orange juice and grapefruit juice, are rich sources of flavonoids, folate, and vitamin C (20). Commercial fresh orange juice has high content of vitamin C (54mg/100 ml juice), flavonoids (10.7 mg²/100 ml juice) and carotenoids (423.9 µg²/100 ml juice) (21). In this study snack form corn flour was produced. The processed snacks, commercial snacks and commercial snacks + orange juice were evaluated in rats to ascertain effects on lipid profile, haematogenic characteristics, liver and kidney functions, glucose, malonaldehyde, weight gain and histological examination of liver and kidney tissues.

2. Materials and methods

Commercial karate (jellio) was purchased from El-Gawhara Company for Food Industries. Corn flour was purchased from National Company for Corn Products, El-Asher min Ramadan, Egypt. Orange was purchased from the local market, Shibin El-Kom, Egypt.

2.1 Preparation of processed snacks

Corn flour was used to prepare the corn based snacks by using twin screw extruder (Wenger TX52) with the following extrusion information: Feed screw speed 13 rpm, preconditioner speed, 150rpm; extruder shaft speed, 340 rpm; Head temperature, 133°C; Head pressure, 1600 psi. The products were sprayed using corn oil (15%) on the extruded product after it was dried at 125 °C for 4-5 min.

2.2 Experimental Design

Twenty four weanling male albino rats, Sprague drawly strain, weighing $45-50 \pm 5$ g were purchased from Helwan farm. The rats were housed individually in cages and fed basal diet for one week for adaptation. The basal diet consisted of 100 g/kg corn oil; 140 g/kg casein; 40 g/kg mineral mixture, USP XIV; 10 g/kg vitamin mixture; 3 g/kg DL-methionine and 2 g/kg choline chloride and 50 g/kg fiber and corn starch 505 g/kg (22). The rats were randomly divided into four groups, 6 rats per group. Control group fed basal diet, the other three groups were fed processed snacks, commercial snacks and commercial snacks + 2 ml dally of orange juice (15% of snacks was incorporated into the basal diet at the expense of corn starch content). Body weight was recorded at the beginning and at the end of experimental period. At the end of experimental period (8 weeks), the rats fasted overnight and anaesthetized. Blood sample were collected and aliquots were analyzed to measure the hematological parameters. The remaining blood was centrifuged to obtain serum for determination serum glucose, serum lipid profile (total cholesterol, triglyceride and LDL, HDL, VLDL), kidney functions (urea and creatinine), liver functions (ALT and AST) and malonlaldehyde (MDA).

2.3 Analytical methods:

Total nitrogen content, fat, moisture, and ash were determined according to (23). The carbohydrate was calculated by difference. Serum glucose was estimated according to Rojas et al., (1999). Alanine amino transferase (ALT), aspartate amino transferase (AST) and malonlaldehyde (MDA) were assayed by the methods of (25,26) respectively. Hemoglobin (Hb) red blood cell (RBC) and haematocrit (Ht) in heparinized blood samples were measured using automated hematology analyzer (Sysmex, Kobe, Japan). Urea and creatinine levels were determined according to the method described by (27). Serum total cholesterol, triglyceride (TG) and high density lipoprotein (HDL) were determined by using methods of (28,29,30) respectively. The determination of low density lipoprotein (LDL), very low density lipoprotein (VLDL) and mean corpuscular volume (MCV) were carried out according to the methods of (31) as follows:

$$\text{VLDL} = \frac{\text{TG}}{5}$$

$$\text{LDL} = \text{Total cholesterol} - (\text{HDL} + \text{VLDL})$$

$$\text{MCV} = \frac{\text{HT}}{\text{RBC}} \times 10$$

2.4 Histopathology examinations

Small specimens of the organs liver and kidney were taken from each experimental group, fixed in neutral buffered formalin, dehydrated in ascending concentration of ethanol (70, 80 and 90%), cleared in zylene and embedded in paraffin. Sections of 4–6 μm thickness were prepared and stained with hematoxylin and eosin according to (32).

2.5 Statistical Analysis

The experimental data were subjected to an analysis of variance (ANOVA) for a completely randomized design using a statistical analysis system (33). Duncan's multiple range tests were used to determine the differences among means at the level of 95%

3. 3. Results and Discussion

Table (1) showed the proximate chemical composition of commercial and processed snacks. There were significant ($P \leq 0.05$) differences in fat and carbohydrate between commercial and processed snacks. While no significant ($p > 0.05$) differences were observed among protein, moisture and ash. The commercial snacks had higher content of fat (38.16%) than the processed snacks (15.57%). These results are in agreement with reported by (34) who found that the chemical composition of commercial snacks were 8.8, 35.56, 3.28 and 47.78% for protein, fat, moisture and carbohydrate respectively.

Body weight gain of rats fed basal diet and snacks diets were presented in Table (2). Rats fed commercial snacks diet had the highest ($P \leq 0.05$) final weight as compared to rats fed basal diet, processed snacks diet and commercial snacks diet + orange juice. This effect may be due to the high content of fat in commercial snacks diet. There was no significantly ($P > 0.05$) difference in weight gain between rats fed basal diet and rats fed processed snacks. Rats fed commercial snacks diet and commercial snacks diet + orange juice had higher ($P \leq 0.05$) weight gain than rat fed processed snacks. These results are in agreement with those reported by (6,35,10) they found that there were significant associations between snacks food consumption and increased BMI, increased body weight and a higher probability of being overweight.

Serum lipids profile of rats fed basal and snacks diets were shown in Table (3). There were no significant ($P > 0.05$) differences in cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) between rats fed basal diet and those fed processed snacks. However, cholesterol and LDL were significantly ($P \leq 0.05$) increased in rats fed commercial snacks and commercial snacks + orange juice as compared with rats fed basal and processed

snacks diets. Rats fed processed snacks, commercial snacks and commercial snacks + orange juice had higher ($P \leq 0.05$) triglyceride than those fed basal diet.

Cholesterol, triglyceride, LDL and VLDLc were reduced ($P \leq 0.05$) by supplementation commercial snacks diet with orange juice as source of natural antioxidant. These results are in agreement with those reported by (36) they found that snacks food consumption increases concentration of cholesterol, triglycerides and lipoproteins of low and very low density. The serum LDL and cholesterol decreased by 43% and 32%, respectively after supplementation of hypercholesterolemia rabbits diets with orange juice, (37).

Data presented in Table (4) showed the hemoglobin (Hb), haematocrit (Ht), red blood cell (RBC) and mean corpuscular volume (MCV) of rats fed basal and snacks diets. There were no significant ($P > 0.05$) differences in hemoglobin, haematocrit, red blood cell and mean corpuscular volume in rats fed basal diet and rats fed all type of snacks diets except mean corpuscular volume in rats fed commercial snacks diet which significant ($P \leq 0.05$) decrease. Similar results were reported by (34) who found that there was no significant difference in Hb, Ht, RBC and MCV in children after had commercial snacks and commercial snacks + lemon juice for two months.

Liver and kidney functions of rat fed basal and snack diets were shown in Table (5). Rats fed commercial snacks and commercial snacks + orange juice diets had a higher ($P \leq 0.05$) serum aspartate amino transferase (AST) activity than those fed basal diet, processed snacks diets. However, rats fed commercial snacks diet had a higher ($P \leq 0.05$) serum alanine amino transferase (ALT) activity than those fed basal diet, processed snacks diet and commercial snacks + orange juice diet. There were no significant ($P > 0.05$) differences in ALT and AST enzymes between rats fed basal diet and those fed processed snacks diet. Also, there were no significant ($P > 0.05$) differences in ALT enzyme among rats fed basal diet, processed snacks diet and commercial snacks + orange juice diet. These results are in agreement with (38) who reported that after consumption three meals a day of junk food restaurant for 1 month liver ALT enzyme peaked at 290 u/l from baseline value 20 u/l.

On the other hand, there was no significantly ($P > 0.05$) difference in creatinine between rats fed basal and all snacks diets. Rats fed commercial snacks diet had a higher ($P \leq 0.05$) urea than those fed

basal diet, processed snacks diet and commercial snacks diet + orange juice. Supplementation of commercial snacks diet with orange juice resulted in a significant ($P \leq 0.05$) decrease in ALT enzyme and urea however, AST enzyme and creatinine was not affected.

Malonaldehyde (MDA) and glucose of rats fed basal and snacks diets were presented in Table (6). There was no significant ($P > 0.05$) difference in glucose between rats fed basal diet and other snacks diets. There was no significant ($P > 0.05$) difference in malonaldehyde between rats fed basal diet and processed snacks diet. However, malonaldehyde was affected ($P \leq 0.05$) by commercial snacks diet and commercial snacks diet + orange juice. Rats fed commercial snacks diet and commercial snacks diet + orange juice had a higher value of malonaldehyde than those fed basal diet and processed snacks diet. Supplementation of commercial snacks diet with orange juice resulted in a significant ($P \leq 0.05$) reduction in malonaldehyde concentration. Adequate dietary antioxidant supplementation may be effective in lowering oxidative stress (39). Similar results were reported by (40) who found that ascorbic acid significantly decrease the adverse effect of reactive species such as reactive oxygen and nitrogen species that can cause oxidative damage to macro molecules such as lipids, DNA and proteins which are implicated in chronic diseases.

Figure (1) showed the histological examination of liver tissues of rats fed basal and snacks diets. The liver tissues of rats fed basal diet, processed snacks diet and commercial snacks diet + orange juice showed no histopathological change. However, liver tissue of rats fed commercial snacks diet showed activation of epithelial lining bile duct, portal infiltration with mononuclear leucocytic cells. As well as necrosis and atrophy of some hepatocytes were found.

Figure (2) showed the histological examination of kidney tissues of rats fed basal and snacks diets. The kidney tissues of rats fed basal diet, processed snacks diet and commercial snacks diet + orange juice revealed the normal histology of renal parenchyma. While, kidney tissues of rats fed commercial snacks diet showed granularity of epithelial lining renal tubules, presence of eosinophilic proteinaceous cast in the lumen of some renal tubules associated with atrophy of glomerulus tuft.

Table1: Proximate chemical composition of processed and commercial snacks.

	Protein (%)	Moisture (%)	Fat (%)	Carbohydrate (%)	Ash (%)
Processed snack	8.53 ^a ± 0.15	3.73 ^a ± 0.21	15.57 ^b ± 0.3	68.96 ^a ± .45	3.53 ^a ± 0.15
Commercial snack	8.37 ^a ± .15	4.13 ^a ± 0.15	38.16 ^a ± 1.25	46.2 ^b ± 1.25	3.13 ^a ± 0.21
LSD	0.35	0.41	2.1	2.1	0.41

Means in the same column for each variable with different letters are significantly different ($p \leq 0.05$)

Table 2: Body weight gain of rats fed basal and snacks diets.

Groups variables	Control	Snacks groups			LSD
		Processed Snacks	Commercial Snacks	Commercial snacks + O J ¹	
Initial weight (g)	46 ^a ± 1.7	47.3 ^a ± 1.5	47.67 ^a ± 2.5	47 ^a ± 2.6	4
Final weight (g)	98 ^d ± 12	104 ^c ± 2.6	119.67 ^a ± 4.9	112.67 ^b ± 4.9	5.5
Weight gain (%)	52.39 ^b ± 1.57	54.5 ^b ± 0.64	60.2 ^a ± 1.26	58.3 ^a ± 0.51	2.8

¹ orange juice

Means in the same row for each variable with different letters are significantly different ($p \leq 0.05$)

Table 3: Serum lipid profile of rats fed basal and snacks diets.

Groups variables	control	Snacks groups			LSD
		Processed snacks	Commercial snacks	Commercial snacks + O J ¹	
Cholesterol (mg/dl)	102.99 ^c ± 1.3	104.33 ^c ± 1.5	124.17 ^a ± 1.3	121 ^b ± 2	2.9
Triglyceride (mg/dl)	105.13 ^c ± 1.8	110 ^b ± 3	119.83 ^a ± 1.3	112.7 ^b ± 2.5	4.23
HDL (mg/dl)	41.53 ^a ± 1.6	41.1 ^a ± 0.92	32.63 ^c ± 2.5	37.4 ^b ± 1.4	3.18
LDL (mg/dl)	40.9 ^c ± 1.1	40.9 ^c ± 1.5	67.6 ^a ± 2.1	61.1 ^b ± 0.3	2.65
VLDL (mg/dl)	21.0 ^c ± 0.36	22.3 ^b ± 0.83	23.96 ^a ± 0.25	22.53 ^b ± 0.5	1.0

¹ orange juice

Means in the same row for each variable with different letters are significantly different ($p \leq 0.05$)

Table 4: Hemoglobin, haematocrit, red blood cell and mean corpuscular volume of rats fed basal and snacks diets.

Groups Variables	control	Snacks groups			LSD
		Processed Snacks	Commercial Snacks	Commercial snacks + O J ¹	
Hemoglobin (g/dl)	13.1 ^a ± 0.6	13.3 ^a ± 0.5	13.5 ^a ± 0.5	13.5 ^a ± 0.3	0.92
Haematocrit (%)	47 ^a ± 2	50 ^a ± 2	50.7 ^a ± 0.6	52 ^a ± 3	3.92
Red blood cell (mil/cmm)	6.5 ^a ± 0.3	7 ^a ± 0.2	7.3 ^a ± 0.2	7.2 ^a ± 0.3	0.48
Mean corpuscular volume (fl)	72.32 ^a ± 0.3	71.4 ^a ± 0.82	69.9 ^b ± 0.54	72.2 ^a ± 1.6	1.45

¹ orange juice

Means in the same row for each variable with different letters are significantly different ($p \leq 0.05$)

Table 5: Liver and kidney functions of rats fed basal and snacks diets.

Groups Variables	control	Snacks groups			LSD
		Processed snacks	Commercial snacks	Commercial snacks + O J ¹	
ALT (u/l)	95 ^b ± 2	95.33 ^b ± 3.5	122 ^a ± 2.6	98.33 ^b ± 1.52	4.8
AST (u/l)	44.73 ^b ± 1.3	43.77 ^b ± 1.5	59.67 ^a ± 3	45.63 ^a ± 0.98	3.6
Urea (mg/dl)	19.03 ^b ± 0.42	19.43 ^b ± 0.75	25.16 ^a ± 0.76	20.16 ^b ± 0.76	1.3
Creatinine (mg/dl)	0.73 ^a ± 0.03	0.72 ^a ± 0.02	0.77 ^a ± 0.03	0.75 ^a ± 0.02	0.04

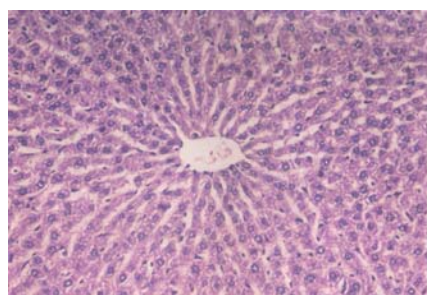
Means in the same row for each variable with different letters are significantly different ($p \leq 0.05$)

Table 6: Malonaldehyde and glucose in rats fed basal and snacks diets.

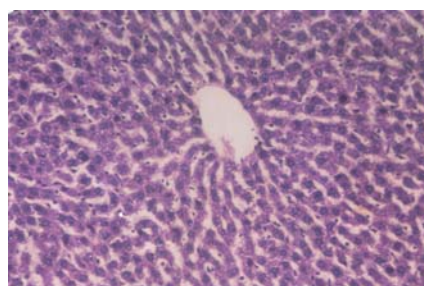
variables	Groups control	Snacks groups			LSD
		Processed snacks	Commercial snacks	Commercial snacks + O J ¹	
MDA ² (n mol/ml)	6.67 ^c ± 0.42	7.3 ^c ± 0.1	13.47 ^a ± 0.55	10.57 ^b ± 0.3	0.72
Glucose (mg/ dl)	110.67 ^a ± 3.96	112.33 ^a ± 4.16	117.33 ^a ± 2.5	113.67 ^a ± 3.2	6.67

¹orange juice, ²Malonaldehyde

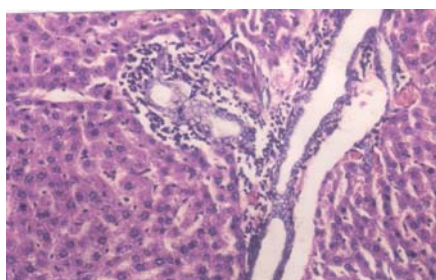
Means in the same row for each variable with different letters are significantly different ($p \leq 0.05$)



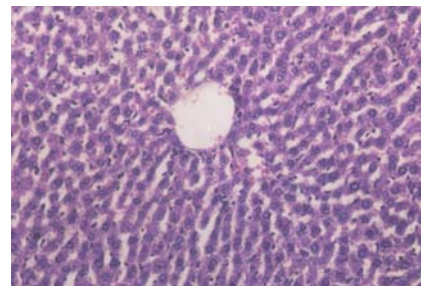
Rats fed basal diet



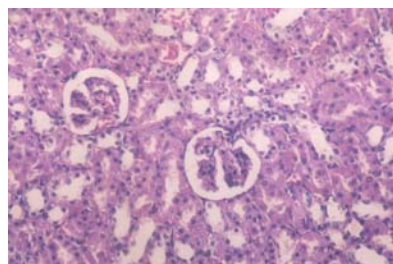
Rats fed processed snacks diet



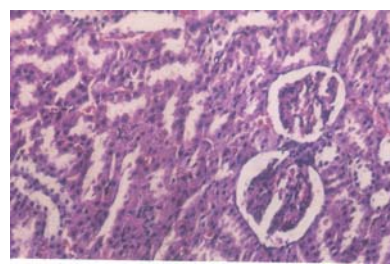
Rats fed commercial snacks diet



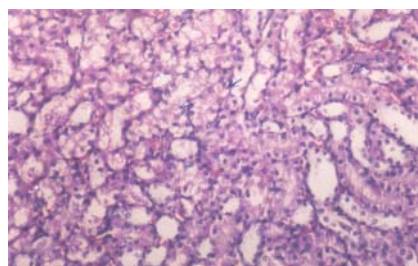
Rats fed commercial snacks diet + O J

Fig (1). Histological examination of liver tissues of weanling rats

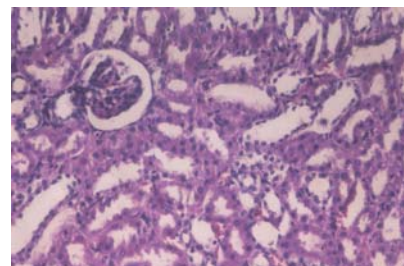
Rats fed basal diet



Rats fed processed snacks diet



Rats fed commercial snacks diet



Rats fed commercial snacks diet + O J

Fig (2). Histological examination of kidney tissues of weanling rats

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