EFFECT OF ROYAL JELLY ON SOME ORGANS OF YOUNG RATS SUBJECTED TO LOW PROTEIN LOW FAT REGIMEN

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It has been noticed that young people in poor countries usually follow regimen, which based on low protein - low fat diet. Since dietary protein is expensive, so they could not have their requirement of it. In this study young rats were fed low protein low fat diet for 21 days. Then, one third of animals were sacrificed while others were divided into two groups. One of this groups was fed on a balanced diet and the other was fed on balanced diet supplemented with Royal Jelly for 30 days.

Significant decreases were found in liver total protein, total lipids of heart, kidney, muscles and total cholesterol of heart, kidney, muscles and brain. On the other hand, a significant increase was recorded in liver total lipids and cholesterol. Other parameters were not affected during this period. From this study, it is clear that both royal jelly and balanced diet have a good effect in ameliorating the resulted hazards of this diet.

Introduction:

Malnutrition is the most important health problem in developing countries in the world today. Protein malnutrition (PEM) occur characteristically in children under five years, where ever the diet is poor in protein and energy. Death rate in these children may be 20-50 times more than the rate in rich and prosperous communities in Europe and North America. No age is immune, but in older persons the disease is much lesser frequent, because protein and energy requirements are relatively reduced as age advances(1). In Egypt, malnutrition particularly among young children and their mothers is an important problem(2) .

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Malnutrition affects all ages across the entire lifespan. From the moment of conception, through out fetal life. Iodine, folate and intrauterine nutrition have a profound influence on development, growth, morbidity, mortality, not only in utero and in early infancy, but on morbidity, physical and mental capacity through out life, including the development of diet-related nutritional calorie deficiencies later in life [3].

Malnutrition is known to cause biochemical alterations in the brains of humans and other animals, and its effects appear to be specific for various gene products[4]. Brain proteins of mature rats are preferentially spared during starvation. Protein turnover declines very early in response to food deprivation, and the decrease in protein synthesis result mainly from a decrease in ribosomal activity[5]. Skeletal muscles, liver protein content and glycogen reduced greatly in the malnourished rats. When adequate diet is restored, liver quickly regains original protein content. Skeletal muscles regain weight and protein content gradually[6].

Royal jelly has moderate antitumor activity [7]. Promote wound healing, [8] and stimulate or inhibit various aspects of immune function[9].

Many people in the developing countries suffer from obesity. They follow many kinds of diets for weight loss, but their diets are usually poor in protein and fats. So this study was planned to investigate the effect of low protein-low fat regimen on some physiological parameters of animals and to identify the possible hazards of such regimen. Also, to assess the role of balanced diet only and balanced diet with Royal Jelly as recovery agents.

Material and Methods

Thirty male albino rats of similar age and size, weighing 55±5g at the beginning of the experiment, were fed balanced diet for 10 days. After this adaptation period, when their weight reached 80±10g,they were randomly divided into 2 main groups as the following:

(a) Control group (12 rats) fed on a balanced diet containing 10% protein and 10% fat.

(b) Low protein low fat group (18 rats) fed on low protein low fat diet containing 3% protein and 5% olive oil.

The Composition of Control Diet and Low Protein Low Fat Diet

Diets Constituents g%	Control diet [10]	Low protein - low fat diet
Casein	13	3.9
Maize starch	67	76.1
Olive oil	10	5
Mineral mixture[11]	4	4
Vitamin mixture[12]	1	1
Cellulose	5	5

All animals were kept in healthy cages at room temperature. Food and tap water were freely available. After twenty-one days, six individuals from each group were sacrificed. The remainder of regimen group was divided into two groups, one received the balanced diet as that of the control group and the other received the balanced diet and administered with royal jelly for thirty days for recovery.

The amount of royal jelly that given to the individuals was calculated using multiplication factors for dose conversion between different species (The conversion factor from man of 70 kg to rat of 200g is 0.018) according to Paget and Barnes[13].

After 30 days of the recovery period, animals were scarificed and as soon as possible organs were removed. Pieces of each organ were weighed and put in saline to determine cholesterol level, aspartate and alanine aminotransferase activities, or put in 0.1N sodium hydroxide to determine total protein or in concentrated sulphoric acid to determine total lipids. Total lipid was determined according to the method of knight et al.[14]. Cholesterol has been detected according to the method of Allain et al. [15]. Protein was estimated according to Doumas[16]. Aspartate and Alanine transaminases activities were determined according to the method described by Reitman and Frankel[17].

The statistical analysis of the obtained data was done according to Armitage[18] and Lentner et al.[19] . The analysis was revised by Quattro pro for windows program version 2-Microsoft Windows version 7.

Results

The data presented in table (1) revealed a significant decrease ($P<0.05$) in liver protein content in the regimen group after 21 days. On the other hand, total protein content of heart, kidney, brain and muscle did not show significant difference in all groups whether before or after recovery period.

After thirty days of recovery period the liver protein content showed nonsignificant change in the regimen group received balanced diet with Royal Jelly. While that received balanced diet only was still indicate "significant" decrease ($P < 0.05$) as shown in table (1).

Table (1)
The Effect of Low Protein Low Fat Regimen on Total Protein Content (mg/g Tissue) in Different Organs After 21 Days of Treatment and 30 Days of Recovery Period in Albino Rats

Criteria		21 Days of Treatment		30 Days of Recovery		
		Control	Regimen group	Control	Balanced diet	Balanced diet and Royal Jelly
Heart	$\overline{X}$	87.000	87.017	89.383	87.000	87.950
	S.E	1.222	1.430	1.405	1.913	2.008
	P		---		---	---
Kidney	$\overline{X}$	64.167	59.950	62.733	59.450	59.133
	S.E	1.440	2.109	1.041	2.134	2.299
	P		---		---	---
Brain	$\overline{X}$	11.483	11.867	11.883	11.600	12.083
	S.E	1.336	1.562	1.721	0.759	0.878
	P		---		---	---
Liver	$\overline{X}$	52.783	37.500	54.700	45.167	54.700
	S.E	2.305	6.334	3.078	1.723	3.078
	P		<0.05		<0.05	---
Muscle	$\overline{X}$	12.500	13.917	11.533	11.050	10.583
	S.E	1.900	1.145	2.413	1.365	3.279
	P		---		---	---

A significant decrease ($P < 0.01$) was observed in the total lipid content of heart, kidney and muscle in the regimen group. On the other hand, total lipid content of liver showed a significant increase ($P< 0.01$) in the same group after 21 days of the experiment. While brain content of lipid showed nonsignificant change throughout the time of the experiment in all groups. After thirty days of recovery period, total lipid content in all these major organs did not show statistical differences in all groups when compared with the control group (table 2).

Table 2
Effect of Low Protein -low Fat Regimen on Total Lipid Content (mg /g Tissue) in Different Organs After 21 Days of Regimen and 30 Days of Recovery Period in Albino Rats

Criteria		21 Days of Treatment		30 Days of Recovery		
		Control	Regimen group	Control	Balanced diet	Balanced diet and Royal Jelly
Heart	$\overline{X}$	52.00	18.00	47.00	53.00	46.00
	S.E	0.704	0.309	0.546	1.1184	0.400
	P		<0. 01		--	--
Kidney	$\overline{X}$	103.00	49.00	99.00	93.00	98.00
	S.E	0.392	0.796	0.873	0.257	0.704
	P		<0.01		--	--
Brain	$\overline{X}$	82.083	80.067	81.150	82.520	81.717
	S.E	2.968	2.191	1.766	2.071	2.595
	P		--		--	--
Liver	$\overline{X}$	22.733	45.167	24.400	25.167	23.500
	S.E	0.926	1.352	0.938	1.078	2.432
	P		<0.01		--	--
Muscle	$\overline{X}$	89.00	23.00	83.00	87.00	87.00
	S.E	0.811	0.326	0.628	0.653	0.372
	P		<0.01		--	--

As shown in table (3), a significant decrease ($P < 0.01$) was indicated in the cholesterol content of heart, kidney, brain and muscle in the regimen group after the treated period. On the contrary, liver showed a significant increase ($P < 0.01$) in its cholesterol content. While no significant change was detected in all the examined organs after the recovery period in the groups treated with balanced diet with Royal Jelly.

Table (3)
Effect of Low Protein - Low Fat Regimen on Cholesterol Content (mg/g Tissue) in Different Organs During 21 Days of Treatment and 30 Days of Recovery Period in Albino Rats

Criteria		21 Days of Treatment		30 Days of Recovery		
		Control	Regimen group	Control	Balanced diet	Balanced diet and Royal Jelly
Heart	$\overline{X}$	9.500	1.830	8.330	7.830	8.500
	S.E	0.102	0.065	0.042	0.070	0.050
	P		<0. 01		--	--
Kidney	$\overline{X}$	20.330	15.175	18.830	18.670	19.670
	S.E	0.076	0.054	0.087	0.080	0.095
	P		<0. 01		--	--
Brain	$\overline{X}$	15.83	5.500	16.000	16.330	15.500
	S.E	0.249	0.159	0.211	0.105	0.138
	P		<0. 01		--	--
Liver	$\overline{X}$	6.500	20.170	6.670	4.500	5.670
	S.E	0.180	0.296	0.194	0.171	0.233
	P		<0.01		--	--
Muscle	$\overline{X}$	11.330	3.500	15.170	14.500	14.670
	S.E	0.095	0.034	0.105	0.163	0.131
	P		<0.01		--	--

Concerning ALT activity, a significant decrease was observed in kidney and liver ($P<0.01$) and muscle ($P<0.05$) after the regimen period. While no significant change was observed in both heart and brain (table 4). On the other hand, AST activity showed a significant decrease in heart ($P<0.05$), kidney and liver ($P<0.01$) only. No significant change was recorded in both brain and muscle when compared with the control group after the treated period (table 5). Both ALT and AST activities showed no significant change after the recovery period in all the tested organs (in rats treated with balanced diet only or balanced diet and Royal Jelly).

Table (4)
Effect of Regimen on ALT (Alanine Aminotransferase) Activity (u/g Tissue) in Different Organs After 21 Days of Treatment and 30 Days of Recovery Period in Albino Rats.

Criteria		21 Days of Treatment		30 Days of Recovery		
		Control	Regimen group	Control	Balanced diet	Balanced diet and Royal Jelly
Heart	$\bar{X}$	6.400	6.400	11.567	12.117	19.000
	S.E	0.581	0.888	0.812	0.819	0.967
	P		--		--	--
Kidney	$\bar{X}$	12.783	7.517	13.667	13.100	16.994
	S.E	0.534	0.193	0.850	0.425	0.667
	P		<0. 01		--	--
Brain	$\bar{X}$	4.153	4.108	6.401	6.533	11.500
	S.E	0.392	0.973	0.276	0.653	0.931
	P				--	-
Liver	$\bar{X}$	16.433	13.983	16.962	16.583	17.000
	S.E	0.107	0.048	0.881	0.671	0.712
	P		<0. 01		--	--
Muscle	$\bar{X}$	13.567	13.281	15.815	15.567	16.117
	S.E	0.107	0.043	0.381	0.749	0.962
	P		<0. 05		--	--

Table (5)
Effect of Low Protein -low Fat Regimen on AST (Aspartate Aminotransferase) Activity (u/g Tissue) in Differentorgans After 21 Days of Treatment and 30 Days of Recovery Period in Albino Rats

Criteria		21 Days of Treatment		30 Days of Recovery Period		
		Control	Regimen group	Control	Balanced diet	Balanced diet and Royal Jelly
Heart	$\bar{X}$	16.917	16.633	18.800	18.700	19.000
	S.E	0.107	0.049	0.900	0.932	0.967
	P		<0. 05		--	--
Kidney	$\bar{X}$	16.200	12.000	16.567	16.555	16.994
	S.E	0.500	0.333	0.667	0.800	0.667
	P		<0. 01		--	--
Brain	$\bar{X}$	10.000	11.000	10.833	11.000	11.500
	S.E	0.601	0.967	0.667	0.567	0.931
	P		--		--	--
Liver	$\bar{X}$	18.333	14.033	19.867	19.867	20.000
	S.E	0.700	0.400	0.733	0.538	0.712
	P		<0. 01		--	--
Muscle	$\bar{X}$	17.000	16.867	16.955	17.017	16.117
	S.E	0.333	0.709	0.798	0.529	0.962
	P		--		--	--

Discussion:

In the present work, liver protein content showed significant decrease in the regimen group. On the other hand, total protein content of heart, kidney, brain and muscles did not show any significant change in all groups, after twenty-one days of regimen.

In agreement with our results, Cherel et al. [20] and Viana et al.[21] revealed that brain proteins of mature rats are preferentially spared during starvation. Protein turnover declines very early in response to

food deprivation, and the decrease in protein synthesis result mainly from a decrease in ribosomal activity. Heymsfield et al. [22] stated that when protein-energy malnutrition (PEM) is the result of underlying disease, organs and tissues begin to resemble brain, in that the protein pools within them are critical for survival. This is a rather marked contrast to the uncomplicated semistarved rat in which heart, muscles and kidney showed significant atrophy[23].

Heymsfield et al. [24] investigated that skeletal muscle, liver protein content and glycogen reduced greatly in the malnourished rats. When adequate diet is restored, liver quickly regains original protein content. Skeletal muscle regain weight and protein content gradually. On the contrary, brain loses no mass, therefore brain would be a very poor marker of the protein-energy malnutrition (PEM) process, and liver would be a sensitive marker in the early phases of PEM development and recovery.

The present work revealed that total lipid and cholesterol content of heart, kidney and muscle decreased significantly in the regimen group. On the contrary, total lipid and cholesterol content of liver was increased significantly in the same group. While total lipid of brain recorded nonsignificant change after the regimen period. These results were concomitant with that of Williams and Hurlebaus [25] who reported that liver content of lipid and cholesterol increased significantly in the sample protein deficiency group after 8 weeks. Furthermore, the protein deficiency produced an increase in the liver neutral glycerides. In the present work, the increase in liver lipid content may be a reflection of infiltration of fats in the liver as indicated by EL-Nabawy et al.[26] and Waterlow [27]. They reported that fatty infiltration of the liver is a constant feature and it is suggested to be sometimes the cause of death in KWO.

Transaminases (AST and ALT) represent a group of enzymes that are present within the cytoplasm of the living cells with the highest concentrations of ALT found in liver tissue, lower concentrations present in heart, muscle and relatively small amounts are present in brain, kidney and serum [28]. AST was found to have its highest concentration in a vriety of tissues including liver, kidney, skeletal and cardiac muscles[29].

Muscle ALT and heart AST indicated a significant decrease in low protein-low fat group after 21 days of malnutrition. The decreasing of AST and ALT activities may be correlated to the decreased biosynthesis of these enzymes as a result of lowered protein intake, and / or with the sustained increase in their serum levels due to their loss from the tissues as found in a previous study. In contrary to our results, Mimura et al. [30] revealed that protein deprivation causes a significant reduction in the oxidation of valine by muscle but not by liver. So, transaminase activity in muscles is increased.

It's clear that, after the regimen period, rats fed balanced diet with royal jelly showed more improvement, faster returning back to normal, and more closer to the control individuals than those received balanced diet only.

From the present results, it is well recommended to use royal jelly to help revealing from sever malnutrition. Further investigations must be done to illustrate the mechanism of royal jelly on malnourished cases, and compare between it and other natural product on such cases.

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ملخـص

تأثير غذاء ملكات النحل على بعض أعضاء صغار فئران التجارب المغذاة على وجبات منخفضة المحتوى من البروتينات والدهون

إيمان هلال وآخرون

يعتمد الشباب فى بعض المجتمعات الفقيرة فى غذائهم علي وجبات غير متكاملة العناصر (ضعيفة المحتوى من البروتينات والدهون اللازمة لنمو الجسم) ، مما يؤدى بهم إلى أمراض سوء التغذية .

لذلك يهدف هذا البحث إلى دراسة تأثير الوجبات التى لاتحتوى على كميات كافية من البروتينات والدهون على محتوى الكبد والمخ والكلى والقلب والعضلات لفئران التجارب من بعض المكونات الحيوية للجسم ، ومدى إمكانية استخدام غذاء ملكات النحل "الرويال چيلى" للعلاج .

وقد أثبتت الدراسة وجود نقص حاد فى محتوى الكبد من البروتينات الكلية ومحتوى القلب والكلى والعضلات من الدهون الكلية ومحتوى القلب والكلى والمخ والعضلات من الكوليسترول ، وإن هناك ارتفاع فى محتوى الكبد من الدهون الكلية والكوليسترول للفئران التى تغذت على وجبات ضعيفة المحتوى من البروتينات والدهون .

وقد تحسنت هذه الأوضاع كثيرا بإعطاء وجبات كاملة العناصر مضافا إليها غذاء ملكات النحل "الرويال چيلى" لمدة ٣٠ يوم .