

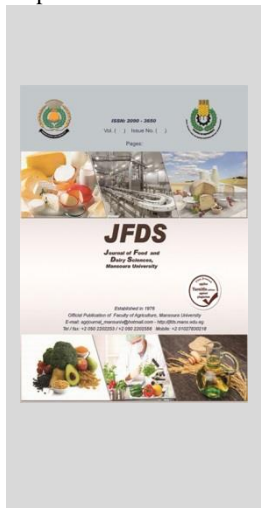
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### Effect of Fermented Kefir Consumption Mixed with Different Cereals Formula on Obese Rats

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#### ABSTRACT

This study investigates the ant obesity effects of fermented Kefir and germinated cereals mixed with fermented Kefir on high fat diet induced obese rats. 25 male rats each weighting  $75 \pm 3$  gram were apportioned to five groups. Group 1 is the negative group. The rest rats were received high saturated fats (34%) in diet for 56 days to become obese rats. Obesity was confirmed by elevation of serum lipid profile and body weight. Group 2 is control +ve group which fed on high fat diet, Kefir group which fed on high fat diet supplemented with kefir at dose of 4ml /rat/day orally, formula 1 group which fed on high fat diet with 20 % of formula 1 (wheat, barley, maize and millet mixture powder melt in 200 ml kefir) and formula 2 group which fed on high fat diet with 20 % of formula 2 (wheat, barley, maize and oat mixture powder melt in 200 ml kefir) for four weeks. This study resulted in a marked improvement of nutritional indicator as kefir Formula 1 and 2 group administration could lower nutritional indicators. However, the administration of kefir, formula 1 and formula 2 could reduce lipids parameters. Consumption of kefir significantly reduced the serum leptin, glucose, insulin resistance and progesterone levels but increased insulin and testosterone levels while consumption of formula 1 and 2 could lower serum leptin and insulin resistance and elevate progesterone and testosterone levels compared to control (+ve) group. It is concluded that germinated cereals mixed with kefir could manage treat obesity.

**Keywords:** Obesity, Kefir, Nutritional and Lipids Parameters, Hormones, Rats

#### INTRODUCTION

Obesity has become a serious problem in the past few years. It is linked to accumulation body fat with over growth and the amplification of adipose tissue because a maladjustment between energy consumption and expenditures (Must *et al.*, 1999). Obesity is often associated with a variety of chronic diseases such as hyperlipidemia, diabetes mellitus, hypertension coronary artery disease, and certain cancers (Kopelman 2000). Although a number of antiobesity pharmacological drugs to the treatment of obesity, more recent trials have focused on screening natural sources that could reduce body weight with minimal side effects (Mayer *et al.*, 2009).

The most popular cereals around the world are wheat, maize, barley and oat that have many functional nutrients and related to reduction the risk factors of many diseases. Whole wheat and maize have influence in resistance atherosclerosis, diabetes, hyperlipidemia, and obesity due to contain effective nutrients and phytochemicals (Higgins 2004 and Fred *et al.*, 2013). Also, barley is an old grain in the Middle East. The renewed interest comes from its ability to produce positive effects on diseases such as glycemic responses after eat, blood lipids and blood pressure (Ames and Rhymer, 2008). Barley has relation to satiety due to its low Glycemic index values and high fiber content. Not only that but also there are synergistic effect between barley  $\beta$ -glucan and reducing blood cholesterol (Shatwan *et al.*, 2013).

Oats and millet have an ability to prevent massive body weight loss seem to be due to its ability to reduce

hyperglycemia and resistance obesity (Zdunczyk *et al.*, 2006; Queenan *et al.*, 2007 and Thilagavathi and Kanchana 2017). Germination process increases the bioactive compounds such as protein, lipid and carbohydrate through modifying its chemical composition (Rumiyati *et al.*, 2012).

Kefir consists of bacteria and yeast species that have symbiotic metabolic activity. It is fermented milk that has an acidic flavor, yeasty aroma, creamy consistency (Witthuhn *et al.*, 2005).

The process of fermentation is carried out by microorganisms through the secretion of enzymes, such as proteases, amylases and lipases which change the polysaccharides, proteins and lipids to nontoxic products attractive to the human consumer (Steinkraus, 1997). Production of many vitamins and minerals are increased during kefir fermentation and lactic acid bacteria in kefir have beneficial effects such as management obesity (Ikeda *et al.*, 2014).

This study was carried out to investigate the antiobesity effects of fermented Kefir and germinated cereals mixed with fermented Kefir on high fat diet induced obese rats by measuring nutritional and serum lipids indicators, some biochemical parameters as leptin, glucose, insulin and hormonal changes and antioxidant enzymes.

#### MATERIALS AND METHODS

##### Materials:

-Experimental cereals Whole wheat, barley, maize, millet, oat and skimmed milk were obtained from local market in

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Mansoura, Egypt except oat was obtained from Agricultural Research center, Giza, Egypt.  
 -Kefir grains were purchased from Agricultural Research Center. Giza, Egypt.

**Methods:**

The experimental cereals were immersed in water (1:5) for 6 hours then spread them in wet blotting paper on a flat surface at room temperature for 4 days and continue in spraying them with water every 12 h. At the end of germination process, wheat was dried in air oven at 45 °C then grinding into powder.

Fifty gram of kefir grains was added to 1000 ml of skimmed milk which was previously boiled and cooled to 37 °C and incubated for 16 h in anaerobic conditions and with ventilation every four hours to stimulate yeast growth (Celso et al., 2005). After incubation, pasteurized brine (brine preparation: 5 g of salt dissolved in 650 mL of water and pasteurized at 90 °C for 15 min) was added to coagulate kefir and mixed together, thoroughly in order to produce a functional beverage free ethanol and the count of lactic acid bacillus will also increase accordance with industrial production of kefir in Iran. Kefir beverage is stored at 4 °C until use (Tayyebbeh et al., 2017).

Formula 1 composed wheat, barley, maize and millet mixture powder (70:10:10:10 %w/w) melt in 200ml kefir while formula 2 composed of wheat, barley, maize and oat mixture powder (70:10:10:10 %w/w) melt in 200ml kefir.

**Experimental animals:**

Twenty five albino male rats of Sprague - Dawley strain, weighted 75± 3g, were obtained from Faculty of Pharmacy, Mansoura University, Egypt. Rats were fed on standard diet (NRC, 1995) and kept for a week for adaption. Food and water were introduced. Five rats were received the basal diet daily as a negative group. The rest rats were received high saturated fats (34%) in diet for 56 days to become obese rats. Obesity was confirmed by elevation of serum lipid profile and body weight (Wang et al., 2010). The obese rats were classified into the following:

- Control positive group that obese rats were fed on high fat diet, given 1 ml of normal saline daily using stomach tube for four weeks.
- Kefir group that obese rats fed on high fat diet supplemented with kefir at dose of 4ml /rat/day by oral administration for four weeks.

- Formula 1 group that obese rats were fed on high fat diet with 20 % of formula 1 for four weeks.
- Formula 2 group that obese rats were fed on high fat diet with 20 % of formula 2 for four weeks.

Daily food intake and weekly body weight were recorded. At the end of the experiment, rats were anaesthetized by diethyl ether to obtain blood, white adipose tissue and brown adipose tissue samples. Serum cholesterol (CHO), triglyceride (TG) and high density lipoprotein cholesterol (HDLc) were estimated according to Richmond (1973), Buccolo and David (1973) and Grodon and Amer (1977), respectively. Total testosterone and progesterone hormones, leptin, insulin and glucose levels were estimated by methods of Orczyk et al., (1974), Zanato et al., (1994), Iwase et al., (2000) and Missouri et al., (2001), respectively.

Food efficiency ratio (FER), protein intake, protein efficiency ratio (PER) and very low density lipoprotein cholesterol (VLDL-c) were calculated at the end of experiment accordance to Chapman et al., (1959), Lee and Nieman (1996) and Friedewald et al., (1972). Atherogenic index was measured by two equations (cholesterol/HDL-c and LDL-c /HDLc) according to Castelli and Levitar, (1977). Insulin resistant was also calculated (fasting glucose × fasting insulin/405) according to Gungor et al., (2004).

Statistical analysis was made by SPSS computer software according to (Artimage and Berry 1987). The calculation accrued by analysis of variance and follow up LSD (SPSS) Computer program variation

**RESULTS AND DISCUSSION**

Data in table (1) illustrated that the final body weight, weight gain, weight gain%, daily food intake and daily protein intake of the control (+ve) group were significantly greater but FER and PER were significantly lower than those of the control (-ve) group. Kefir administration and formula 1 administration suppressed the elevation of nutritional indicator of final body weight, weight gain%, daily food intake and daily protein intake and increase of FER and PER compared to control (+ve) group while formula 2 administrations could lower all the nutritional indicators compared with control (+ve) group.

**Table 1. Nutritional indicators of experimental rats fed on cereals fortified with fermented kefir.**

Groups variables	Initial weight (g)	Final Weight (g)	Weight Gain(g)	Weight Gain%	Daily Food intake	F E R	Daily protein intake	P E R
Control (-ve)	152.01± 2.92	169.80 ±9.41	17.80 ±1.15	10.53 ± 1.80	18.19 ±1.02	0.034 ±0.0004	3.63 ±0.01	0.175 ±0.006
Cntrol (+ve)	196.01 ±7.48	217.46± 18.22	21.46 ± 2.43	14.40 ±1.78	25.05 ±3.15	0.030 ±.0003	5.01 ±0.03	0.152 ±0.007
kefir	200.48± 16.53	179.01 ±3.63	-21.48 ±1.70	-10.61 ±1.17	20.01 ±2.02	0.038 ±0.0007	4.02 ±0.004	0.190 ±0.004
Formula1	197.66± 14.11	179.28 ±4.01	-18.38 ±2.70	-10.61 ±1.76	20.29 ±1.18	0.032 ±0.0002	4.05 ±.005	0.162 ±0.002
Formula2	182.49± 14.33	170.13 ±5.62	-12.36 ±1.62	-7.26 ±0.05	19.66 ±1.34	0.022 ±0.0008	3.93 ±0.08	0.112 ±0.005

Significant with control group \*p< 0.05 \*\* P< 0.01 \*\*\*P< 0.001.

Mean values in each column having different letter (a, b, c) are significantly different at P< 0.05.

(-ve) control negative group, (+ve) control positive group, (kefir) kefir group (formula 1) germinated cereals wheat, barley, maize and millet mixed with kefir, (formula 2) germinated cereal wheat, barley, maize and oat mixed with kefir .

In agreement with these results, Kefir is nice beverage for individuals whose seeks to obtain ideal weight. This are important for athletes, not only that but also good for small babies and preschoolers. It considered safe beverage against diseases and quick weight gain. Many researchers have been done on it to assure healthy side(Zaheer *et al.*, 2013). Viscosity-forming capacity of Oat has been suggested to be crucial for its effect on satiety-related attributes (Slavin and Green, 2007). Millet cereals have an ability to prevent massive body weight gain seem to be due to its ability to reduce hyperglycemia (Thilagavathi and Kanchana 2017).Bryn C Taylor *et al.*, (2020)said that the consumers for fermented foods get linolenic acid through the metabolism of these foods, linolenic acid promotes public health.María José *et al.*,(2020)have demonstrated thatfermented foods decrease body weight in rats through increasing leptin level, energy expenditure, lipolysis, satiety and decreasingghrelin, adiponectin levels

Our data in table (2) showed that rats fed on high fat diet only (control +ve) group exhibited significantly higher TC, TG, LDLc,VLDLcand atherogenic indexes (LDLc/HDLc& CHO/HDLc)and lower HDLc compared with control -ve group. However, the administration of kefir, formula1 and formula 2 could reduce the elevated parameters to near normal levels and increase HDLc compared with control +ve group.

The obtained results were agreed with those reported by many authors.Diets contain oat improved hypercholesterolemia by increasing the excretions of fecal bile acids, and this improvement due to β-glucan and the lipids and proteins. Oat proteins decreased serum cholesterol and LDL.c contents due to their low Lysine/Arginin and Methionin/Glycine ratio. Oleic acid, linoleic, vitamin E and plant sterols accounted for the hypocholesterolemic effects of oat lipids (Lina *et al.*, 2014). Millet cereals have hypolipidemic effects due to contain high levels of complex carbohydrates, fibre and resistant starch. Someof millet benefits are attributed to polyphenol and fiber contents. It also contains calcium and has anti atherosclerogenic effects(Mathanghi and Sudha 2012 andThilagavathi and Kanchana 2017).On the other side,Glore *et al.*,(1994) Anderson (2003)andSaka *et al.*,(2016)emphasized those there benefits for lowering lipids with oats and oat based products have been reported in animal and human studies.Review of Conor *et al.*, (2019) examines and assesses that Kefir contain microorganisms that have been associated with more health benefits; such as cholesterol metabolism.Results obtained from recent studies by kui and his colleagues (2020),the summary of these findings is that fermented foods intake has a relationship with a lower risk of cardiovascular diseases.

**Table 2. Lipids profile and atherogenic indexof rats fed on cereals fortified with fermented kefir consumption.**

Groups variables	T.C (mg/dl)	T.G (mg/dl)	HDLc (mg/dl)	LDLc (mg/dl)	VLDLc (mg/dl)	LDLc/ HDLc	CHO/ HDLc
Control (-ve)	b 67.77 ±2.06	b 48.51 ±1.54	a 45.01 ±3.10	ab 95.87 ±4.47	b 9.7 ±0.92	C 0.77 ±0.11	cd 1.50 ±1.08
Control (+ve)	a *** 97.00 ±2.06	a *** 86.59 ±1.98	d *** 20.53 ±0.50	a 100.21 ±5.27	a ** 17.32 ±1.39	a *** 4.14 ±0.06	a *** 4.72 ±0.06
kefir	b 68.87 ±2.06	b 48.60 ±1.65	b ** 40.33 ±1.47	c *** 88.89 ±2.41	b 9.7 ±0.41	b* 1.94 ±0.43	c 1.71 ±0.13
Formula1	bc 66.69 ±3.59	bC 44.77 ±2.27	c *** 31.11 ±0.15	c *** 88.89 ±0.70	bc 8.95 ±0.45	b* 1.43 ±0.18	b ** 2.15 ±0.16
Formula2	b c 66.68 ±0.32	b 46.92 ±4.41	c *** 29.76 ±0.87	c *** 87.06 ±0.68	b 9.38 ±0.87	b* 1.52 ±0.14	b ** 2.24 ±0.13

Significant with control group \*p< 0.05 \*\* P< 0.01 \*\*\*P< 0.001.

Mean values in each column having different letter (a, b, c.) are significantly different at P< 0.05.(-ve) control negative group, (+ve) control positive group, (kefir) kefir group (formula 1) germinated cereals wheat, barley, maize and millet mixed with kefir, (formula 2) germinated cereal wheat, barley, maize and oat mixed with kefir

In our study, the blood leptin, glucose, insulin resistance and progesterone levels were increased but insulin and testosterone were decreased by consumption of high fat diet compared with control -ve group. It is noticeable that consumption of kefir significantly reduced the serum leptin, glucose, insulin resistance and progesterone levels but insulin and testosterone levels increased compared to control +ve group consumption of formula 1 could lower serum leptin and insulin resistance and elevate, insulin progesterone and testosterone levels while consumption of formula 2 had ability to lower serum leptin,glucose and insulin resistance and increase progesterone and testosterone hormones compared to control +ve group(Table 3).

It is well known that leptin is a key fat-derived regulator of appetite and energy expenditure, normally correlate positively with the extent of the TG stores in adipocytes(Friedman and Halaas 1998).Kefir was used as a

functional food for diabetes therapy through maintaining plasma glucose levels, lowering insulin resistant, increasing antioxidant enzymes activity and improving in pancreatic β-cells (Rosa, 2014 and Nurliyani 2015).Millet are high in resistant starch, these products release sugar slowly in the blood and also decrease the glucose absorption. The slow digestion of resistant starch has implications in controlling glucose release. Millet cereals have hypoglycemic effects due to fibre and complex carbohydrates (Nugent 2005 and Thilagavathi and Kanchana 2017). Oat is abundant in β-glucan, which could lower the glycemic index and control of postprandial glycemia (Kalra and Jood 2000 andRuiling *et al.*, 2011). Rats fed on fermented food showed an ameliorative effect in testosterone level (Mona *et al.*, 2014). Kefir is a beverage made by kefir cereals that change milk proteins into many peptides with healthy effects, such as calcium-absorption and positive effects on testosterone levels

(Ahmed *et al.*, 2013 and Min 2015).Review of Conor *et al.*, (2019)examines and assesses that Kefir contain microorganisms that have been associated with more health benefits; from them cholesterol metabolism which considered very important to regulate hormones in the body;antioxidant effects and antidiabetic effects.

These results indicate that administration of kefir or formula1 and formula 2 resulting in improved nutritional indicators and serum lipid profiles and reduce of serum leptin, glucose and insulin resistance and increase progesterone and testosterone hormones.

**Table 3. Leptin, insulin, glucose, insulin resistant and two hormones testosterone and progesterone of rats fed on cereals fortified with fermented kefir consumption.**

Groups variables	Leptin (ng/ml)	Insulin (mg/dl)	Glucose (mg/dl)	Insulin resistant (µU/mL)	Testosterone (ng/ml)	Progesterone (mg/dl)
Control (-ve)	c 2.03 ±0.40	a 3.36 ±0.15	b 136.00 ±3.67	d 0.12 ±0.03	a 3.25 ±0.18	e 1.20 ±0.02
Control (+ve)	a *** 6.08 ±0.54	c *** 1.28 ±0.33	a ** 146.40 ±2.30	a *** 2.82 ±0.11	d *** 1.23 ±0.07	b *** 4.50 ±0.04
kefir	c d 1.23 ±0.76	b ** 2.86 ±0.01	b 136.20 ±6.34	b *** 0.95 ±0.15	ab 2.91 ±0.05	d ** 1.98 ±0.05
Formula1	cd* 1.95 ±0.45	b ** 2.98 ±0.05	a ** 147.40 ±6.61	b *** 0.86 ±0.12	b ** 2.27 ±0.88	a *** 5.49 ±0.05
Formula2	b** 3.28 ±0.78	c *** 1.32 ±0.02	b 137.20 ±4.43	c *** 0.45 ±0.11	C *** 2.02 ±0.68	a *** 5.28 ±0.03

Significant with control group \*p< 0.05 \*\* P< 0.01 \*\*\*P< 0.001.

Mean values in each column having different letter (a, b, c.) are significantly different at P< 0.05.(-ve) control negative group, (+ve) control positive group, (kefir) kefir group (formula 1) germinated cereals wheat, barley, maize and millet mixed with kefir, (formula 2) germinated cereal wheat, barley, maize and oat mixed with kefir

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### تأثير تناول الكفير المخمر المخلوط بتركيبات مختلفة من الحبوب على الفئران البدينة عبد الغنى محمود عبدالغنى ، لبنى احمد شلبياية و فوزية رفعت عبد الحميد قسم الاقتصاد المنزلي - كلية التربية النوعية - جامعة المنصورة - مصر

يهدف هذا البحث إلى دراسة تأثير الكفير المخمر والحبوب المخلوطة فيه على الفئران المصابة بالسمنة. حيث أجريت الدراسة على 25 ذكور الفئران يتراوح وزنها  $75 \pm 3$  في البداية تم تقسيمهم إلى خمس مجموعات كل مجموعة تحتوي على خمسة فئران . المجموعة الأولى هي المجموعة الضابطة السالبة. وباقي الفئران تناولت وجبات تحتوي على نسبة عالية من الدهون المشبعة (34%) لمدة 56 يوماً لإصابتهم بالسمنة وتم التأكد من اصابتهم بالسمنة بارتفاع وزن الجسم وتحليل مستوى دهون الدم قبل بدء التجربة وبعد اصابتهم بالسمنة تم تقسيم الفئران إلى المجموعة الضابطة الموجبة والمجموعة التي تتغذى على الكفير بجرعة 4 مل / الفئران / يوم، ومجموعة الترتكيبه الأولى تتغذى على وجبه عاليه الدهون مع 20 % من مخلوط مسحوق كل من (القمح والشعير والذرة والدخن)مخلوط مع 200 مللي من الكفير ومجموعة الترتكيبه الثانيه تتغذى على وجبه عاليه الدهون مع 20 % من مخلوط مسحوق (القمح والشعير والذرة الشوفان مع 200 مللي من الكفير) لمدة أربعة أسابيع. وأسفر نتائج الدراسة على وجود تحسن معنوي فيمؤشر التغذية، حيث اظهرت مجموعات الترتكيبه الأولى والثانية خفض معنوي في الوزن النهائي ونسبه الوزن المكتسب والمتناول من الطعام اليومي والبروتين اليومي وتناول الكفير مع الترتكيبه الأولى والثانية اظهر انخفاض معنوي في مستويات الدهون المرتفعة ومؤشر تصلب الشرايين حتى وصل إلى المستويات الطبيعيه . كما أدى تناول الكفير إلى انخفاض معنوي في مستوى هرمون الليبتين والجلوكوز ومقاومة الأنسولين ومقاومة البروجيسترول ومستوي هرمون البروجيسترول كما أدى تناول كل من الترتكيبه الأولى والثانية إلى انخفاض معنوي فيمستوى الليبتين ومقاومة الأنسولين وارتفاع معنوي في مستوى البروجيسترول والتستوستيرون بالمقارنة بالمجموعة الضابطة الموجبه. ومن ذلك نستنتج أن الكفير المخمر و الحبوب الممزوجة بالكفير لهم تأثير علاجي للسمنة وخفض احتمال تصلب الشرايين.نوصى الدراسة بزيادة تناول الكفير في السوق المصرى بشكل اكبر و اضافته للنظام الغذائى وتقديم الأغذية المتخمرة بوجه عام إلى الأطفال والنبتيين فهو غذاء امن وكذلك تسليط الضوء على الحبوب المتخمرة واهميتها لصحة الانسان.

الكلمات الداله:- السمنة - الكفير - المؤشرات الغذائيه ودهون الدم - الهرمونات- الفئران