Anti-obesity and hypoglycemic effects of kefir drink with some grains in experimental animals

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Abstract:

This research investigated the effect of consumption of fermented kefir only or mixed with cereals (wheat, barley, maize and Mung bean) on nutritional and biochemical parameters on obese rats. Twenty albino male rats were classified into a negative control (five rats received the stander diet daily) and obese rats (received high fat diet 34% for 56 days). The obese rats were classified into three group, control (+ve) group and tow treated groups with kefir "4 ml/day/rat by stomach tube" and formula "20% of cereals mixed with 200ml kefir" for four weeks. The nutritional results revealed that Kefir and formula (cereals mixed with fermented kefir) administration suppressed the elevation of nutritional indicator of final body weight of obese rats. Biochemical results showed that rat group fed on kefir or formula had a significant improvement of lipid profile, atherogenic indexes and liver and kidney function values. Also these groups had significant lower value of leptin, glucose, insulin resistance, progesterone and malondialdehyde and higher values of insulin, superoxide dismutase, glutathione peroxides and testosterone compared with control (+ve) group. It is concluded that consumption of fermented kefir either separately or mixed with the most popular cereals have ability to manage over weight and could lower side effects of obesity so it is recommended to consume kefir and the examined formula in weight reduction strategies.

Keywords: Overweight, fermented kefir beverage, cereals, lipid, glucose and rats

INTRODUCTION:

Obesity was a serious problem and still increased. It is an abnormal accumulation of body fat due to maladjustment between energy

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consumption**.** Obesity is associated with development of health risks such as hypertension, coronary artery disease, type 2 diabetes and liver diseases **(Mayer et al., 2009)**. The high-fat diet enriched in saturated fat and lack of unsaturated fat facilitates the prevalence of obesity worldwide **(Li et al., 2008)**.

Many strategies are used to manage obesity as diet control, medications, exercise and lastly surgery. The exercise and diet control are very difficult to be carried on. Medication has many side effects. Recently, a new approach to control this medical disorder is administration plants and for developing safe and effective drugs **(Yin et al., 2014)**. The human gut contains an enormous number of gut microbiota, consists of at least 10^{14} bacteria including anaerobic bacteria.. Obesity is linked to changes in the composition and metabolic function of the gut microbiota **(Neish., 2009)**.

Kefir beverage is prepared from fermentation of kefir grain in milk that contains acetic acid bacteria, lactic acid bacteria, and yeasts found in polysaccharides and proteins a complex matrix. Kefir has health benefits as anti-inflammatory, pro-digestive and antitumor effects (**Lee et al., 2007)**. Kefir also has several biological activities in immune system, reduction of lactose intolerance symptoms and anti-oxidative **(Ho et al., 2013)**. Therefore, in the present study kefir was fermented from kefir grain and formula of most popular consumed cereal as wheat, barley, maize and Mung bean was prepared.

The aims of this study were to evaluate the anti-obesity and glycemic effects of kefir and examined cereals mixed with kefir in rats consumed high fat diet.

MATERIAL AND METHODS:

All cereals (wheat, barley, maize and skimmed milk) were supplied from local market. Mung bean and Kefir cereals were obtained from Agricultural research center Giza, Egypt. The fat of meat was obtained from Butcher.

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, then dried in air oven at 45 ^{O}C and grinding into powder.

Fifty gm. of kefir cereals was added to 1000 ml of skimmed milk which was previously boiled and cooled to 37 ºc 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 (5 g of salt dissolved in 650 mL of water and pasteurized at 90 ºC for15 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 and stored at 4°C until use (**Tayyebeh et al., 2017)**. Formula consisted of experimental cereals as wheat, barley, maize and mung bean mixture (70:10:10:10 %w/w) fermented with 200ml kefir.

Twenty albino male rats, weighted 152± 3g, were obtained from Faculty of Pharmacy Mansoura University, Egypt .The standard diet was prepared according to **NRC, (1995)**. The high fat diet was standard diet had 34% fats **(Wang et al., 2010 and Abd El-Ghany et al., 2017)**. After adaptation period, rats were classified into a negative control (five rats received the standard diet daily). The rest rats were received high fat diet for 56 days to induce obesity that confirmed by increase in serum lipid profile and body weight. The obesity rats were classified into non treated positive control group, kefir treated group (4 ml/day/rat by stomach tube) and formula treated group (fed on high fat diet with 20% of experimental cereals mixed with 200ml kefir) for four weeks. Daily food intake and weekly body weight were recorded. After scarifying rats, blood samples were collected.

Serum cholesterol (CHO), triglyceride (TG) and high density lipoprotein cholesterol (HDLc) were estimated according to **Richmond (1973), Buccolo and David (1973) and Grodon & 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 Sapin et al., (2001), respectively. ALT and AST (alanine and aspartate aminotransferase) enzymes activity was measured accordance to the method of **Pappas,** (**1989)**. Serum albumin, creatinine and uric acid were measured accordance to **Doumas et al., (1971), Young,** (**2001)** and **Fossati et al.,** (**1980)**, respectively. Superoxide dismutase, glutathione peroxides activity and malondialdehyde (SOD, GPX and MDA) in serum were estimated according a way of **Nishikimi et al., (1972)**, **Paglia and Valentine (1967**) and **Ohkawa et al., (1979),** respectively

Feed 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)**. Athrogenic 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. The calculation accrued by analysis of variance and follow up LSD (SPSS) Computer program variation **(Artimage and Berry 1987)**.

RESULT 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) rat group were significantly higher but FER and PER were significantly lower than those of the control (-ve) group. Kefir administration suppressed the elevation of nutritional indicator of final body weight, weight gain, weight gain%, daily food intake and daily protein intake and increase of FER and PER while formula administrations could lower final body weight, weight gain, weight gain%, FER and PER daily and non-significant difference of food intake and daily protein intake compared with control (+ve) group.

Table (1): Effect of kefir and formula of cereals mixed with fermented kefir on nutritional indicators in obese rat groups.

Groups Variables	Initial weight (g)	Final Weight (g)	Weight Gain (g)	Weight Gain %	Daily Food intake	FER	Daily protein intake	Protein Efficiency ratio
Control (-ve)	b 152.01± 2.92	bc 169.80 ±9.41	b 17.80 ±1.15	b 10.53 ± 1.80	cd 18.19 ±1.02	b 0.034 ±0.0004	c 3.63 ±0.01	b 0.175 ±0.006
Control (+ve)	a 217.46± 18.22	a 196.01 ±7.48	a 21.46 ± 2.43	a 14.40 ±1 .78	a 25.05 ±3.15	c 0.030 ±.0003	a 5.01 ±0.03	c 0.152 ±0.00 7
kefir	a 200.48± 16.53	b 179.01 ±3 .63	c -21.48 ±1.70	b 10.61 ±1.17	bc 20.01 ±2.02	a 0.038 ±0.0007	b 4.02 ±0.004	a 0.190 ±0.004
Formula	a 192.29± 14.87	b 174.47 ± 7.65	b -17.82 ±1.66	b 10.21 ±3.63	ab 23.93 ±2.07	d 0.027 ±0.0001	ab 4.78 ±0.008	d 0.133 ±0.005

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

Biochemical parameters:-

Data in table 2 illustrated that obese control + ve rat group had a significant increase in T.C, T.G, LDLc and VLDLc values and lower of HDLc that elevated atherogenic indexes compared with control (-ve). Rat group fed on kefir or formula (cereals mixed with fermented kefir) had a significant decrease in T.C, T.G, LDLc, VLDLc and atherogenic indexes values and elevated HDLc compared with control (+ve)group.

Table (2): Effect of kefir and formula of cereals mixed with fermented kefir on lipids profile and atherogenic index in obese rat groups.

Groups Variables	**T.C (mg/dl)**	**T.G (mg/dl)**	**HDLc (mg/dl)**	**LDLc (mg/dl)**	**VLDLc (mg/dl)**	**LDLc/HDLc**	**CHO/HDL**
Control (-ve)	b 67.77 ±2.06	b 48.51 ±1.54	a 45.01 ±3.10	b 95.87 ±3.47	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
Formula	b 67.89 ±3.45	c 41.01 ±0.47	c 25.06 ±1 .65	c 87.75 ±3.47	c 8.20 ±0.49	c 0.77 ±0.02	b 2.71 ±0.19

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

Data in table 3 illustrated that obese control +ve rat group had a significant increase in leptin, glucose and insulin resistance values and lower of insulin while rat group fed on kefir showed lower in leptin and insulin and higher value of insulin resistance and non-significant difference of glucose but rat group fed on formula (cereals mixed with fermented kefir) showed lower in insulin and higher value of insulin resistance and non-significant difference of leptin and glucose compared with control (-ve) group. Rat groups fed on kefir or formula had significant decrease of leptin, glucose and insulin resistance values and increase of insulin compared with control (+ve) group.

Table (3): Effect of kefir and formula of cereals mixed with fermented kefir on leptin, insulin, and glucose and insulin resistant in obese rat groups.

Groups Variables	**Leptin (ng/ml)**	**Insulin (mg/dl)**	**Glucose (mg/dl)**	**Insulin resistant (µU/mL)**
Control (-ve)	b 2.03 ±0.40	a 3.36 ±0.15	b 136.00±3.67	d 0.12±0.03
Control (+ve)	a 6.08±0.54	c 1.28±0.33	a 146.40±2.30	a 2.82±0.11
kefir	c 1.23±0.76	b 2.86±0.01	b 136.20±6.34	b 0.95±0.15
Formula	b 2.48±0.38	b 2.01±0.39	bc 130.40±5.72	c 0.64±0.11

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

Data in table 4 illustrated that obese control + ve rat group had a significant increase in AST, ALT, bilirubin, creatinine and uric acid values and lower of albumin value while rat group fed on kefir showed only significant lower of ALT enzyme but rat group fed on formula showed lower in ALT, bilirubin and creatinine values compared with control (-ve) group. Rat groups fed on kefir or formula had a significant lower value of in AST, ALT, bilirubin, creatinine and uric acid and higher value of albumin compared with control (+ve) group.

Table (4): Effect of kefir and formula of cereals mixed with fermented kefir on liver and kidney function in obese rat groups.

Groups variables	AST (U/L)	ALT (U/L)	Albumin (g/dl)	Bilirubin (g/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control (-ve)	b 43.60 ±2.30	b 53.57 ±0.35	a 3.69 ±0.25	b 1.30 ±0.07	b 1.68 ±0.04	b 1.49 ±0.45
Control (+ve)	a *** 79.00 ±8.06	a*** 73.76 ±9.76	b *** 1.57 ±0.39	a*** 3.63 ±0.08	a*** 2.99 ±0.08	a*** 3.83 ±0.15
kefir	b 47.36 ±3.45	c** 44.04 ±6.03	a 4.05 ±0.03	b 1.01 ±0.04	b 1.00 ±0.00	b 1.55 ±0.33
Formula	b 46.81 ±6.38	c** 42.19 ±2.31	a 3.85 ±0.28	c* 0.59 ±0.06	c * 0.80 ±0.00	b 1.24 ±0.17

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

The obese control (+) ve rat group had a significant decrease in testosterone and increase of progesterone value while rat group fed on kefir showed non significant difference of testosterone and higher value of progesterone but rat group fed on formula showed lower testosterone and increase of progesterone value compared with control (-ve) group. Rat groups fed on kefir or formula had a significant increase in testosterone and decrease of progesterone compared with control (+ve) group as found in table (5)

Table (5): Effect of kefir and formula of cereals mixed with fermented kefir on testosterone and progesterone in obese rat groups.

Groups variables	**Testosterone (ng/ml)**	**Progesterone (mg/dl)**
Control(-ve)	a 3.25±0.18	d 1.20 ±0.02
control(+ve)	d 1.23±0.07	a 4.50±0.04
kefir	ab 2.91±0.05	bc 1.98±0.05
Formula	bc 2.22 ±0.01	b 2.12±0.01

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

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

Data in table 6 illustrated that obese control + ve rat group had a significant lower in SOD and GPX values and higher of MDA value while rat group fed on kefir and formula showed a non-significant difference of SOD and lower in GPX values and higher value of MDA compared with control (-ve) group. Rat groups fed on kefir or formula had significant increase of SOD and GPX values and lower value of MDA compared with control (+ve) group.

Table (6): Effect of kefir and formula of cereals mixed with fermented kefir SOD, GPX and MDA in obese rat groups.

Groups variables	SOD	GPX	MDA
Control(-ve)	a 6.97±0.02	a 9.23±0.05	cd 6.38±0.26
control(+ve)	c 1.32±0.04	c 3.50±0.53	a 15.69±0.31
kefir	a 5.63±0.05	b 7.14±0.14	bc 8.86±1.12
Formula	ab 4.92±0.01	b 6.09±0.07	b 10.84±0.30

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

Discussion

To demonstrate the effect of the administration of kefir and different cereals and the status of nutritional , glycemic and lipid metabolism, obese rats consumed high-fat diet (34%fat) , characterized by a significant elevation of final weight, weight gain, serum total cholesterol, LDLc, TG in serum and atherogenic index indexes. These results were agreed with **Mayer et al., (2009).** Consumption of kefir could improve these results and that agreed with results of **Maeda et al., (2004) and Rosa (2014).** Kefir contains a mixed microflora of limited to a kefir grains so played a role in reducing serum lipids so is considered health benefit **(Liu et al., 2006)**. Probiotics strains in kefir could reduce serum triglyceride and total cholesterol, and the improve of atherogenic indexes by bacterial cells as it assimilate cholesterol, reduce cholesterol reabsorption, increases cholesterol excretion and increases cholesterol uptake by low-density lipoprotein receptor pathway in the liver as a compensatory response **(Gill and Guarner 2004 and Abd El-Ghany et al., 2020)**. Obesity is characterized by excess storage of triglycerides in adipose tissue that produces adverse health consequences that is associated with non-alcoholic fatty liver

disease and also an imbalance between energy intake and expenditure. Administration of kefir improves fatty liver syndrome by inhibiting the lipogenesis pathway in leptin-deficient knockout mice **(Tilg et al., 2009 and Kim et al., 2015)**. Obesity is associated with liver and kidney disease as it provokes inflammation and oxidative stress and linked to diabetes and hypertension diseases. Administration of kefir improves biomarkers associated with kidney and liver injury (ALT, AST, creatinine and urea) **(Wanchai et al., 2017).** Administration of kefir to obese rats could reduce fat accumulation and maintain the blood glucose level as adjust the gut microbiota by elevation of Lactobacillus /Lactococcus and yeast and lowering of microbial induced obesity **(Abd El-Ghany et al., 2012 and Lin et al., 2016**). Insulin resistance is main cause of type 2 diabetes and is characteristic of obesity. An accumulation of fat in the liver produces simple hepatic steatosis and progresses toward inflammation with fibrosis **(Racette et al., 2003)**. Regular kefir administration could prevent high fat diet that induced obesity in obese rats. Kefir significantly reduces lipid accumulation by down-regulation of adipogenic transcription factors, which play a critical role in adipocyte differentiation **(Chen et al., 2014a).** Testosterone hormone is essential for the development and maintenance of secondary sexual characteristics in men as well maintenance of spermatogenesis. progesterone is a female hormone, but males need progesterone to produce testosterone and produced from adrenal glands and testes. There is a strong relationship between levels of testosterone and progesterone in the seminal plasma of semen decrease sperm motility **(Kiran and Shrivastav 2007)**. Rats fed on probiotic food showed an ameliorative effect in testosterone level **(Mona et al., 2014).**

Kefir contains abundant natural bioactive peptides and exhibits a variety of biological activities, such as angiotensin-converting enzyme inhibitory, antithrombotic, mineral binding, immunomodulating, antimicrobial, and anti-oxidative effects that explained the improvement of antioxidant and glycemic indicators **(Ebner et al.,** 2015 **and Nurliyani., 2015).** Administration of kefir improves fatty liver syndrome by inhibiting the lipogenesis pathway in leptin-deficient knockout mice. Probiotics could

reduce the inflammatory response which improves insulin resistance in diabetes **(Lye et al., 2009 and Kim et al., 2015).** Many possible mechanisms of hypoglycemic effect of probiotics as it affected gut bacteria to create glucagon-like peptide-l and insulinotropic polypeptides to stimulate uptake of glucose in muscle and liver glycogenesis. Consumption of kefir in diabetic patients could lower the fasting blood glucose and HbA1C levels **(Al-Salami et al., 2008 and Ostadrahimi et al., 2015)**. Furthermore, the obtained results showed synergistic effect of administration of kefir and cereals which related to content of nutritional elements, fibers, antioxidant. Wheat grain consists of the bran, endosperm, and germ parts. The bran consists of fibers, antioxidants, vitamins like B and E, and minerals like iron, copper, zinc and magnesium **(Piironen 2013)**. Whole wheat phytochemicals, beta-sitosterol (lowers cholesterol), dietary fibers, resistant starch, anti-nutrients, such as phytic acids, and tannin have been shown to lower risks of many disease **(Marangoni and Poli 2010)**. In rats fed on whole wheat, there were no significant structural changes in liver **(Jinshan et al., 2015).** As barley has relation to satiety due to its low glycemic index values and high fiber content and synergistic effect between barley β-glucan and reducing blood cholesterol**.** Barley is a cereal high in antioxidant acts as a co-factor for enzymes involved in the glucose metabolism and insulin secretion **(Khalaf and Mohamed, 2008)**. Maize impedes lipogenesis in white adipose tissue and decreases the accumulation of triacylglycerol in liver and white adipose tissue due to anthocyanins **(Tsuda et al., 2003 and Abd El-Ghany, et al., 2012).** Maize has influence in resistance atherosclerosis, hyperlipidemia, diabetes and obesity due to contain effective nutrients and phytochemicals **(Higgins, 2004)**. Mung bean is one of the major grain legumes which contain potential agents for reducing serum lipids suitable for patients with hypercholesterolemia as reported in experimental animal models studies **(Tang et al., 2014 -----).** Mung beans consumption significantly reduced plasma triglyceride level due to its γ-aminobutyric acid which has a potent effect on improve glucose tolerance and also have many antioxidants that act as myocardial preservation agents by regulating cholesterol levels. Flavonoids of Mung

beans have high free-radical scavenging abilities**(Chung et al., 2011, Nurliyani., 2015 and Bai et al., 2016)**. Several researches have concluded that mung bean cereals may be control glucose level (**Reynolds et al., 2006 and Chen et al., 2014b).** Rats fed on processed Mung beans showed significant lower in serum insulin and glucose. The antidiabetic effect of mung beans may be due to its high fiber and low glycemic index **(Liyanage et al., 2018).** Antioxidants in cereals act as myocardial preservation agents by regulating reversing damage to blood vessels, and lowering inflammation **(Chung et al., 2011and Bai et al., 2016)**

Based on the results of the present study, administrations of kefir and experimental cereals have ability to improve the adverse nutritional and biochemical effect resulting from obesity. It is recommended to introduce kefir and examined cereals as regimen strategy in treatment of obesity.

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التأثيرات المضادة للسمنة وجلوكوز الدم لمشروب الكفير مع بعض الحبوب في حيوانات التجارب

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الملخص العربي:

يهدف البحث إلى دراسة تأثير تناول الكفير المخمرمنفرد أو مخلوط مع حبوب كل من (القمح والشعير والذرة والفاصوليا) على مؤشرات التغذية ووظائف الكبد والكلى ونشاط انزيمات مضادات الأكسدة وبعض الهرمونات (الأنسولين والتستوستيرون والبروجسترون) على الفئران البدينة. تم تقسيم عشرين من ذكور الفئران البيضاء إلى مجموعة الكنترول السالبة(خمسة فئران تغذت علي النظام الغذائي الأساسي يوميًا) وخمسة عشرة من الفئران التي تغذت علي النظام الغذائي عالي الدهون ٣٤٪ لمدة ٥٦ يوم للاصابة بالسمنة و.تم تقسيم الفئران البدينة إلى مجموعة الكنترول الموجب التي تغذت علي النظام الغذائي عالي الدهون ومجموعة تغذت علي تغذت علي النظام الغذائي عالي الدهون مع الكفير بجرعة ٤ مل / يوم / فأر عن طريق أنبوب المعدة ومجموعة تغذت علي تغذت علي النظام الغذائي عالي الدهون مع ٢٠٪ من الحبوب المختبرة المخلوطة بـ ٢٠٠ مل من الكفير) لمدة أربعة أسابيع. أظهرت النتائج الغذائية أن تناول الكفير اوالتركيبة من الحبوب المخلوطة بالكفير المخمر أدى إلى تثبيط ارتفاع المؤشر الغذائي لوزن الجسم النهائي وزيادة الوزن ونسبة زيادة الوزن لدى الفئران البدينة. وأظهرت النتائج البيوكيميائية أن مجموعة الفئران التي تغذت على الكفير أو تركيبة الحبوب المخلوطة بالكفير المخمر اظهرت انخفاض معنوي دهون الدم ومؤشرات تصلب الشرايين وقيم وظائف الكبد والكلى. كما أظهرت هذه المجموعات انخفاضً معنوي في قيم اللبتين والجلوكوز ومقاومة الأنسولين والبروجسترون والمالونديالدهيد وارتفاع معنوي لكل من هرمون الأنسولين و التستوستيرون والانزيمات المضادة للاكسدة كسوبر أكسيد ديسميوتاز وبيروكسيد الجلوتاثيون وذلك بالمقارنة بمجموعة الكنترول الموجب .وخلصت الدراسة إلى أن تناول الكفير المخمر منفرد أو مخلوطً مع الحبوب المختبرة كان لهم القدرة على خفض الوزن الزائد و الآثار الجانبية للسمنة لذلك توصى الدراسة بتناول الكفير وتركيبة الجبوب المختبرة مع الكفير في استراتيجيات النظام الغذائي لعلاج السمنة .

الكلمات المفتاحية: الوزن الزائد، مشروبات الكفير المخمرة، الحبوب، الفئران، دهون وجلوكوزالدم

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