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Possible Kefir Biological Effects: 4: Effect of Kefir Beverage on The Histopathological and Macroscopical Changes in Adipose Tissue of High Fat-Fed STZ- Induced Diabetic Male Wistar Rat

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ABSTRACT

This study was designed to investigate the effect of kefir consumptio common dairy fermented product on keep the bodyweight balanced on high streptozotocin-induced diabetic wistar rats which fed with a daily kefir gavage also to examine any effects regards the abdominal circumference (AC) and bo mass; observed results compared to normal male rats; Experiments were carried 60 albino male rats, with age 8 weeks, weighing about 220-250 g, after the ada period, male rats were divided randomly into two experiments by six g experiment I included 3 non-diabetic ones and experiment II included thre induced diabetes groups. The groups were fed as follows: group 1 received a st diet and served as control. Group 2 was fed on a standard diet and kef ml/animal/day by gavage). Group 3 received a high-fat diet and kefi ml/animal/day by gavage). The diabetic males of groups A, B and C were fe high-fat diet. Group B received in addition kefir (0.7 ml/animal/day by gavage), group C was injected additionally with insulin (0.76 UI/200 mg BW/day); groups have the access to the drinking water all the time.; the bodyweight of n and diabetic rat males was determined on 1st-treatment day,-and every week un end the experiment plan (5 weeks) then all the groups were sacrificed with mea the AC and weighting the fat mass; Summarizing it could be said; through all th plan (5 weeks), all the six animal groups showed a significant increase in the body weight with a normal level in experiment I unless in group 3 which fed on and kefir, while in experiment II kefir helps the diabetic treated group to not weight compared to the untreated one; similarity kefir neither affect AC nor relat weight.

INTRODUCTION

The Middle East and North Africa region have the highest prevalence of DM in adults. Egypt has a prevalence range from 7.2 to 11% (Sherif and Sumpio, 2015). Now, Egypt is among the 10 "leading" countries in the world in terms of the number of people with DM.

It is estimated that in 2025, DM prevalence will be 13.4%. The urban population within Egypt has a higher prevalence of DM (20%) compared to the rural population (4.9%) with no significant difference between genders (Bos and Agyemang, 2013).

Several studies suggest that measuring islet autoantibodies in relatives of those with type 1 diabetes may identify individuals who are at risk for developing type 1 diabetes. There is evidence to suggest that early diagnosis may limit acute complications *(*Ziegler *et al.,* 2013).

The intestinal microbiota is a relevant therapeutic source for the treatment of different diseases. Although there have been proposed different strategies including pre/probiotics and fecal microbiota transplantation interventions (Ejtahed *et al.,* 2016).

Kefir has been considered a probiotic due to its antioxidant and anti-inflammatory properties (Guven and Gulmez, 2003; Kwon *et al.,* 2008).

In STZ-induced DM, it has been shown that daily administration of kefir caused an improvement in the increased levels of glycemia and glucose tolerance compared to conventional fermented milk (Yadav *et al.,* 2008; Hadisaputro *et al.,* 2012; Giovana *et al.,* 2014).Besides drug treatment for diabetes; in recent years, many efforts have been made on traditional medicines as a complementary therapy in the treatment of diabetes. In this regard, probiotics have been considered in diabetic patients. (Guarner *et al.,* 2005).

MATERAILS AND METHODS

1- Experimental Animals:

White male albino rats (Wistar rat) (Rattus norvegicus) from order Rodentia and family Muridae were used in the present study. Experiments were carried out on 60 albino rats, aged 8 weeks and weighing 220-250 gm. The animals were obtained from the ENVIGO Company, **USA. IACUC Protocol Number (ORA use only): 2017-17.**

Rats were kept in the Lab of Animal Research Facility (LARF) building, University of Idaho, USA, for 1 week under observation before experimentation to exclude any intercurrent infection and to acclimatize the animals to the new conditions. The selected animals were housed (3-4) in polycarbonate cages with softwood chips as bedding at a-temperature of 23 ± 2°C, relative humidity of 50 ± 5% with good ventilation constant light/dark periods of 12 hours (hr.) each. Rats were either fed on standard rodent pellet diet (for groups 1,2) or fed with a high-fat diet (Sirrivasan *et al.,* 2004), (groups (3, A, B, C), drinking tap water was provided at libitum for all groups.

Composition of HFD:

Ingredients Diet	(g/kg)
Powdered NPD	365
Lard	310
Casein	250
Cholesterol	10
Vitamin and mineral mix	60
dl-Methionine	03
Yeast powder	01
Sodium chloride	01

Generally, the protocol followed the general guidelines of animal care. All efforts were made to minimize the number used and their suffering.

2. Induction of Diabetes Mellitus:

Diabetes mellitus was experimentally

induced in overnight fasted male animals by an intraperitoneal (ip) injection of streptozotocin (STZ) at the dose of 45 mg/kg (Judiono *et al.,* 2011; Suharyo *et al.,* 2012; Giovana *et al.,* 2014). Streptozotocin was dissolved in cold 0.01 M citrate buffer, pH 4.5 and always prepared freshly for immediate use within 5 minutes. The normal control group was given a citrate buffer without STZ. The development of diabetes was confirmed after 48 hours – 7 days of STZ injection. The animals with fasting blood glucose levels of more than 200 mg/dl were considered diabetic and included in this study.

3. Animal Grouping:

Male rats were divided into six groups 10 animals each, 3 non-diabetic, and 3 diabetic groups:

Experiment I:

Group 1- Control animals, (negative group) were fed a standard diet plus oral administration of distilled water at a dose of 0.7 ml/animal/day.

Group 2- animals were fed a standard diet and received oral administration of kefir (0.7 ml/animal/day).

Group 3- Animals received a high-fat diet (HFD) and additionally oral administration of kefir (0.7 ml/animal/day).

Experiment II:

Group A- The diabetic group (positive group), was fed HFD and received oral administration of distilled water (0.7 ml/animal/day).

Group B- Diabetic animals received HFD plus oral administration of kefir (0.7 ml/animal/day.

Group C- Diabetic group, fed HFD, was injected insulin (0.76 UI/200 mg BW/day).

By the end of the experimental time of 5 weeks, animals of all groups fasted 4-6 hours, weighted, anesthetized by isoflurane, and sacrificed. The collected blood was centrifuged, and the serum stored at -80 ◦C until use. Tissues for the histological investigations were excised immediately, fixed in Formal saline 4% for and embedded in paraffin.

3. Experimental Studies:

1-Gross Morphology of Adipose Tissue:

Adipose tissue was dissected out and dried on a filter paper. The absolute weight of the organ was determined, and its relative weight was calculated.

2-Histopathological Examination of Adipose Tissue:

Adipose tissue was immediately excised. Small tissue blocks were prepared and fixed in 4% neutral buffered formalin, then transferred to Washington State University, Veterinary School, Pathological lab, Pullman, WA, USA, for complete tissue process, 5 µm sections were stained in specific dyes such as Haematoxylin and eosin stain.

3-Macroscopic Examination (General Morphology of The Body):

a- Body weight (gm)

The body weight was determined on 1st treatment day, and every week until the end of the experiment plan.

b- Weight Gain (gm):

$$\text{Weight gain} = \left(\frac{\text{Final} - \text{Initial}}{\text{Initial}}\right) \text{x}100$$

c- Abdominal Circumference (cm):

The abdominal width was measured at the widest abdominal area at the end of the experiment.

4. Statistical Analysis:

Variables with a normal distribution were expressed as mean ± standard deviation. Variables with no normal distribution were expressed as median (25th - 75th percentile). One- Way ANOVA test was used for comparing groups mean of normally distributed variables. Four multiple comparisons between different groups were done using Post Hoc Tukey test was used. For not normally distributed variables, Kruskal-Wallis 1-way ANOVA test was used. Data were analyzed using SPSS (Statistical Package for Social Science) version 24 software. P value < 0.05 was considered significant.

RESULTS

1-Gross morphology of adipose tissue

The relative fat weight given in table 1 and figure 1 indicated non-significance change between the different groups in both experiments.

Table 1: Relative fat weight (gm) of two experiments (I and II) groups.

Experiments / Comparison	Experiment I			Experiment II		
Groups / Parameter	Group 1	Group 2	Group 3	Group A	Group B	Group C
Relative Fat weight (gm)	1.51 ± 0.09	1.40 ± 0.13	1.72 ± 0.10	1.36 ± 0.11	1.58 ± 0.19	1.65 ± 0.08
Statistical analysis	NS	NS	NS	NS	NS	NS

Data are given as mean ± S.E.; means with the same superscript are not significantly different
** (p<0.01): highly significant; *(p<0.05): significant; NS: non-significant
Group 1: Control animals, (negative group) were fed a standard diet plus oral administration of distilled water
Group 2: Animals were fed a standard diet and received oral administration of kefir
Group 3: Animals received a high-fat diet (HFD) and additionally oral administration of kefir
Group A: Diabetic group (positive group), was fed HFD and received oral administration of distilled water
Group B: Diabetic animals received HFD plus oral administration of kefir
Group C: Diabetic group, fed HFD, was injected insulin

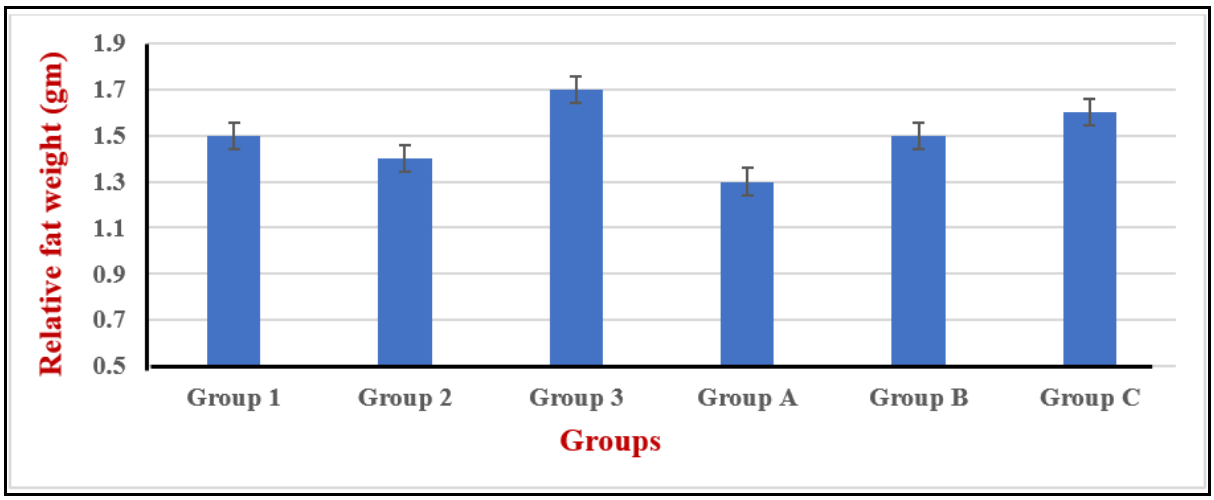

Fig. 1: Relative fat weight (gm) of two experiments (I and II) groups at the end of the experiment

2-Histopathological Examination of Adipose Tissue:

Figure 2 showed the pathological changes in the adipose tissue in the two different experiments.

Experiment I:

Adipose tissue of rat in C-N (group 1) revealed a fully-developed fat cell between pancreatic tissues, adipose tissue of rat in C-N + Kefir (group 2) and adipose tissue of rat CHFD+ Kefir (group 3) revealed full-developed fat cell.

Experiment II:

Similarly, there were no pathological lesions in the adipose tissues all over the experiment ∏ (group A, B, C).

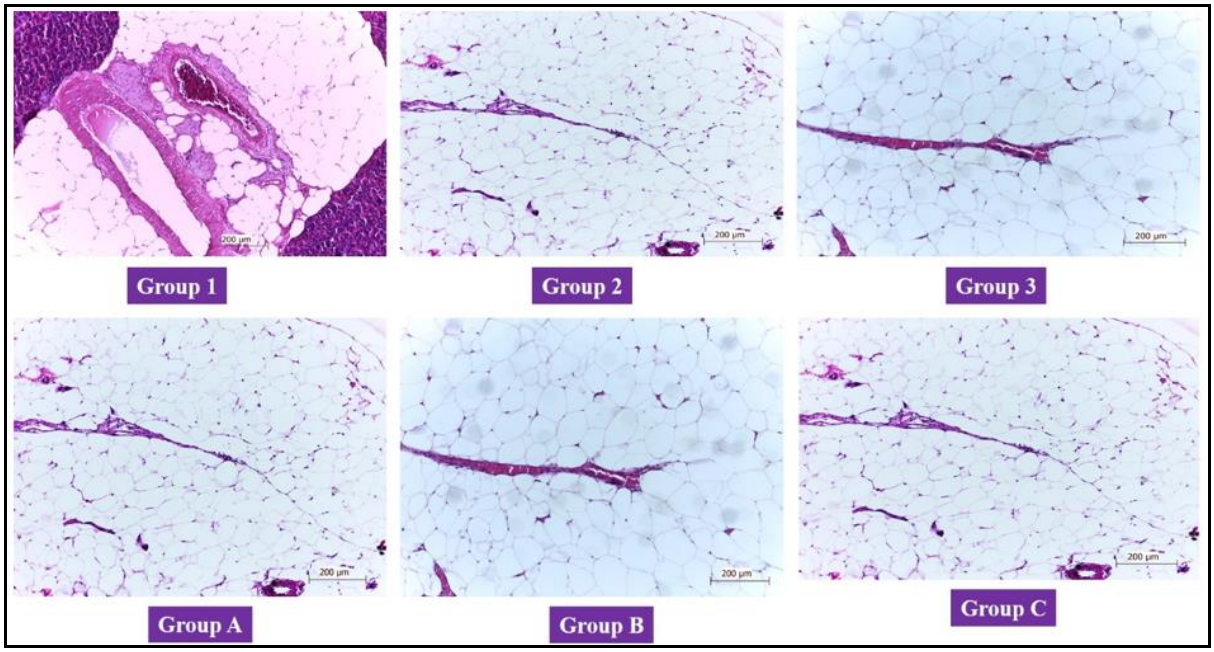

Fig. 2: Pathological changes in the adipose tissue in the different rat groups, H&E, bar= 200 µm.

3-Macroscopic Examination (General Morphology of The Body):

a- Body weight (gm):

The bodyweight of normal and diabetic rat males was determined on 1st treatment day and every week until the end of the experiment plan (5 weeks) represented in table 2 and figure 3.

In experiment I, the bodyweight for the three groups was in the same range from the first day of the experiment and didn't appear any significance between them, through whole the experiment plan the bodyweight of the normal animals increased within the same level and reached 412 to 424 gram.

While in experiment II, the diabetic ones increased with low level and no significant between the groups unless in the last two weeks there was significance between group C and B, group C and A, group 3 and B; the bodyweight of the three groups reached 321 to 383 gram at the end of the experiment.

Summarizing, there was a marked increase in body weight in the six groups in both experiments at the end of the experiment (week 6) as compared to the initial body weight (week 0), the increased level was higher in the normal animals than the diabetic ones.

Table (2): Bodyweight (gm) of two experiments (I and II) at different experimental periods.

Experiments / Comparison Groups / Parameter	Experiment I			Experiment II		
	Group 1	Group 2	Group 3	Group A	Group B	Group C
Body weight at 0 week	237.2 ± 3.27	246.5 ± 3.65	240.1± 4.23	243.3 ± 3.58	237.8 ± 3.12	241.4 ± 1.84
Statistical analysis		NS	NS		NS NS NS	NS
Body weight at week 1	308.4± 8.75	336.9 ±5.37	329.5 ± 5.91	299.3 ± 9.23	297.9 ± 7.39	293.2 ± 4.42
Statistical analysis		**	NS		NS ** NS	NS
Body weight at week 2	330.9 ± 11.01	333.0 ± 7.19	315.8 ±5.70	278.8 ± 12.54	274.5 ± 15.40	260.3 ± 8.98
Statistical analysis		NS	NS		NS ** NS	NS
Body weight at week 3	358.0±12.75	360.4 ± 8.76	346.5 ± 7.56	291.2 ± 14.51	292.4 ± 19.07	317.2 ± 6.69
Statistical analysis		NS	NS		NS ** NS	NS
Body weight at week 4	373.2± 12.75	386.5 ±10.18	359.5 ± 8.12	304.6 ± 16.18	300.9 ± 22.97	335.0 ± 8.51
Statistical analysis		NS	NS		NS ** NS	NS
Body weight at week 5	400.8± 12.34	410.3 ±10.66	394.7 ± 9.82	321.0 ± 18.46	320.7 ± 24.88	370.3 ± 7.51
Statistical analysis		NS	NS		NS ** *	*
Body weight at week 6	419.3± 14.72	424.3 ±10.60	412.9±10.57	321.4 ± 18.77	331.8 ± 28.57	383.9 ± 8.61
Statistical analysis		NS	NS		NS ** *	*

Data are given as mean ± S.E.; means with the same superscript are not significantly different
** ($p<0.01$): highly significant; * ($p<0.05$): significant; NS: non-significant

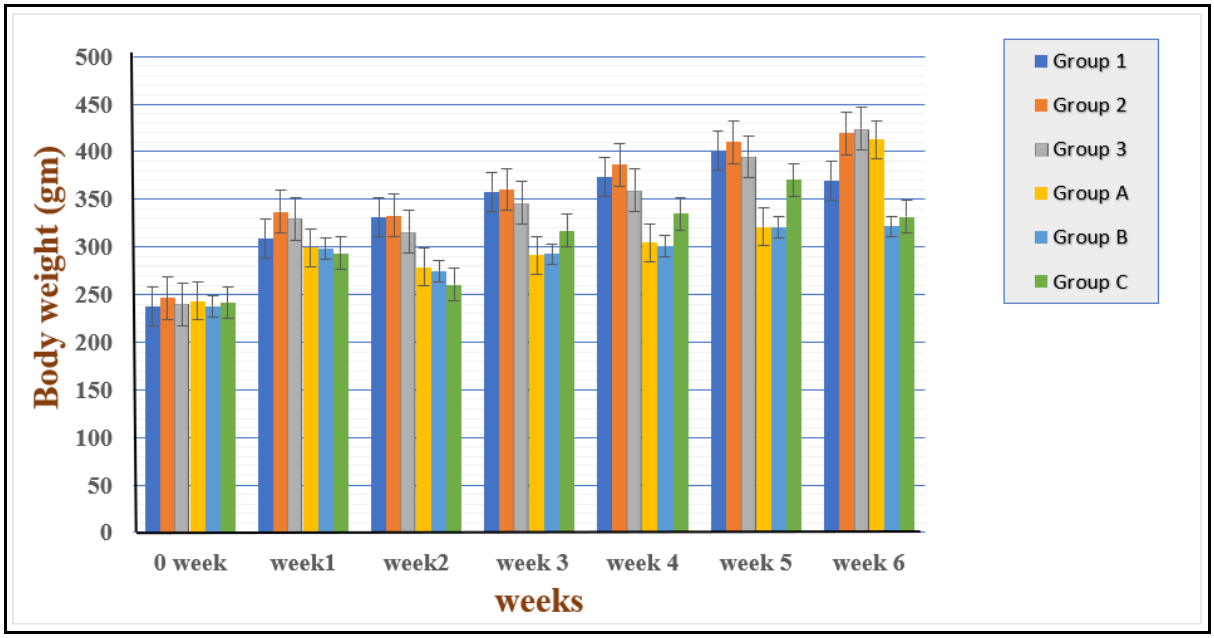

Fig. 3: Bodyweight (gm) of two experiments (I and II) at different experimental periods.

b- Body Weight Gain (gm):

The body weight gain (gm) for each group was represented weekly throughout the experiment, data represented in table 3 and figures 4.

Through the five weeks, each group appeared significant increase in the body weight gain from the beginning of the experiment until the end with variable values, the represented data in experiment I showed non-significance in the body weight gain between the three groups, and through whole the experiment plan the body weight gain of the normal animals increased within the same level and reached 172 to 182 gram.

While in experiment II, the diabetic ones increased with the low rate with no significant between the groups unless in the last week there was significance between group C and A, also group 3 and B through whole the experiment plan; the body weight gain of the three groups reached 91 to 142 gram at the end of the experiment.

Summarizing, the body weight gain rate of the normal animals was more than the diabetic ones, also the body weight gain in group B which treated with kefir was less than the other treated with insulin.

Table 3: Bodyweight gain (gm) of two experiments (I and II) at different experimental periods.

Experiments / Comparison	Experiment I			Experiment II		
Groups / Parameter	Group 1	Group 2	Group 3	Group A	Group B	Group C
Body weight gain at Week1	71.20 ± 7.64	90.40 ± 2.36	89.40 ± 2.47	63.56 ± 7.33	60.10 ± 7.15	51.80 ± 5.38
Statistical analysis		*	NS		NS **	NS NS
Body weight gain at Week 2	93.70 ± 8.65	86.50 ± 4.43	75.70 ± 4.09	52.13 ± 6.66	51.88 ± 11.51	33.13 ± 4.32
Statistical analysis		NS	NS		NS *	NS NS
Body weight gain at Week 3	120.80 ±10.65	113.90 ± 6.46	106.40 ±5.41	65.38 ± 10.29	74.63 ± 14.49	75.80 ± 7.61
Statistical analysis		NS	NS		NS *	NS NS
Body weight gain at Week 4	136.0 ±10.67	140.0 ±7.86	119.40 ± 6.21	80.0 ± 12.82	86.13 ± 18.89	93.60 ± 9.56
Statistical analysis		NS	NS		NS *	NS NS
Body weight gain at Week 5	163.60 ± 10.22	163.80 ± 8.55	154.60 ± 7.59	99.25 ± 14.17	108.25 ± 20.31	128.90 ± 8.44
Statistical analysis		NS	NS		NS **	NS NS
Total body weight gain	182.10 ± 12.46	177.80 ± 8.41	172.80 ± 8.44	91.88 ± 15.84	122.63 ± 23.97	142.50 ± 9.65
Statistical analysis		NS	NS		NS *	NS *

Data are given as mean ± S.E.; means with the same superscript are not significantly different

** ($p<0.01$): highly significant; * ($p<0.05$): significant; NS: non-significant

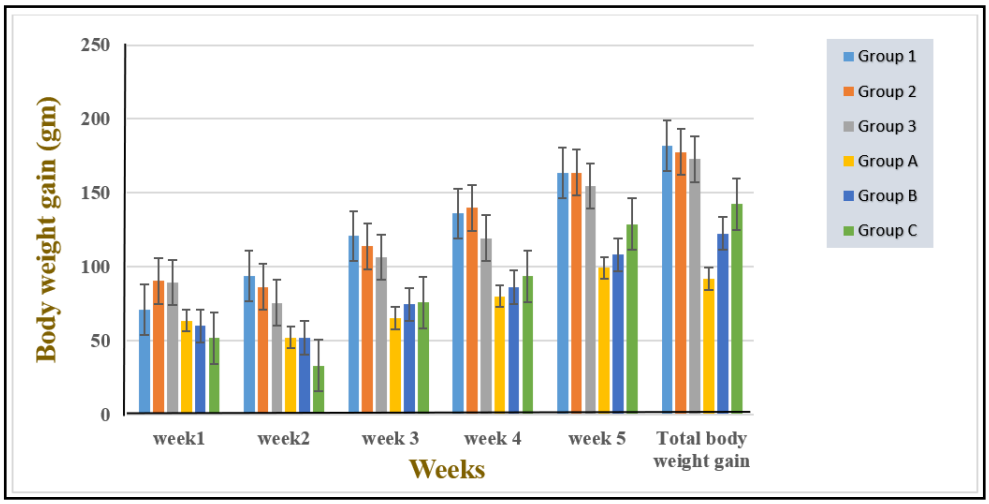

Fig. 4: Body weight gain (gm) of two experiments (I and II) at different experimental periods.

C- Effect on Abdominal Circumference:

The mean abdominal width (cm) was measured at the widest abdominal area for all the animals per each group at the end of the experiment as represented in table 4 and figure 5.

According to the P value, there was no significance between the groups in both experiments I and II, the only significance was between group C and A.

Table 4: Abdominal circumference (cm) of two experiments (I and II) at the end of the experiment**.**

Experiments / Comparison	Experiment I			Experiment II		
Groups / Parameter	Group 1	Group 2	Group 3	Group A	Group B	Group C
Mean abdominal circumference (cm)	17.77 ± 0.31	18.13 ± 0.34	17.23 ± 0.43	16.30 ± 0.31	16.60 ± 0.71	17.75 ± 0.28
Statistical analysis		NS	NS		NS	NS
					NS	
						*

Data are given as mean ± S.E.; means with the same superscript are not significantly different
** (p<0.01): highly significant; *(p<0.05): significant; NS: non-significant

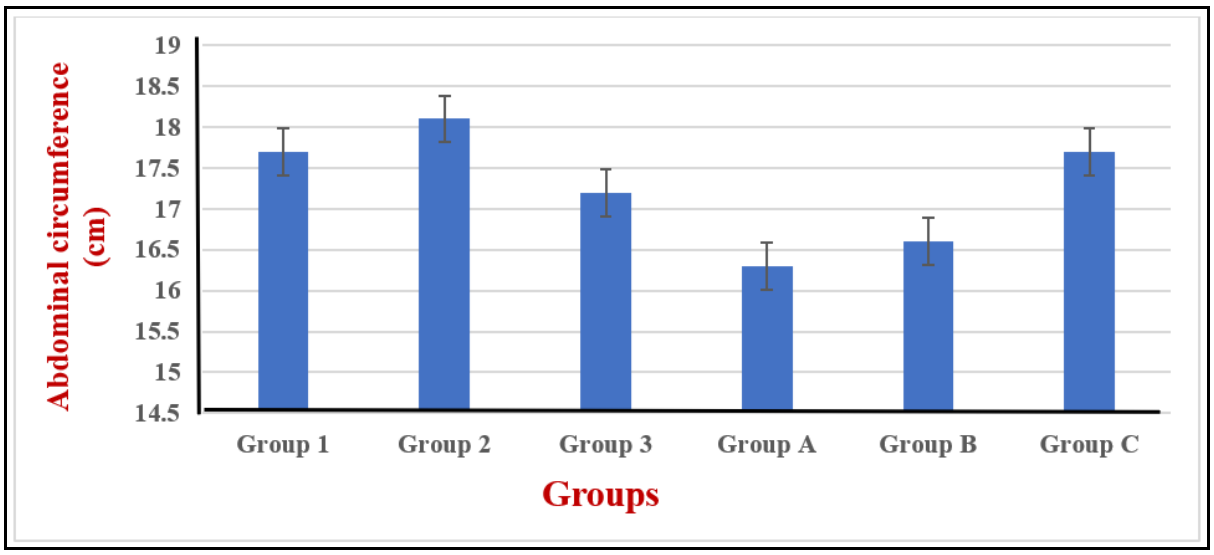

Figure 5: Abdominal circumference (cm) of two experiments (I and II) at the end of the experiment.

DISCUSSION

There is increasing evidence that the gut microbiome has a causal role in obesity and obesity-related metabolic disorders because of its influence on genes that regulate lipid metabolism, energy utilization, and storage (Davis, 2016).

High-fat diet (HFD) was regarded as a major determinant of obesity until it was found that the gut microbiota had a significant role in this phenotype. Gut microbiota plays a vital role in human energy homeostasis and metabolism of fat and cholesterol (Baothman *et al.,* 2016).The gut microbial symbiosis is considered as an underlying reason behind obesity and metabolic syndromes while the balanced and healthy gut microflora is linked with health (Shabana *et al.,* 2018).

Regarding the morphology of the adipose tissue, absolute weight was determined and mean relative weight was calculated; represented data indicated non-significance between the different groups; and that was expected cause of kefir beverage feeding had no effects in increasing fat mass formed, also there were no histopathological changes between the normal animals and diabetic ones.The number of studies included in the meta-analyses of the effects of probiotics on body fat mass and the percentage was low, thus meaning that the latter results should be interpreted with caution.The agreements go with the overall of the seven studies reporting changes in fat mass showed a larger reduction in body fat mass and fat percentage in the intervention groups compared with the control groups, but the difference was non-significant because effect sizes were small (Borgeraas *et al.,* 2018).

However, the studies by Agerholm-Larsen *et al.* (2000) and Lee *et al.,* (2014) were the only studies reporting either increased amounts of fat mass or lower reduction in fat mass in the intervention group compared with the control group,

Urdaneta *et al.,* (2007); Sahin and Yardimci, (2009) showed that using kefir supplemented diet had no significant differences in the weight of the body organs examined.

In experiment I, feeding the normal animals with kefir in group 2 didn't affect the bodyweight of the animals, it remains in the same level as in group 1, however, it was expected changes in the body weight in group 3 which fed on HFD and kefir, so kefir avoided any metabolic disorders.

While in experiment II, the three diabetic ones started with the same bodyweight but through the experiment, there wasn't noticeable gaining weight cause of diabetic complications but we could notice the significance between group C and B; at the end of the experiment, group C treated with insulin showed more bodyweight than group B which treated with kefir and that means kefir had desirable consequences regarding the body weight in the diabetic animals; also it keeps animals accepting weight even their illness symptoms.

The weight gain in the trial groups was an expected result when previous studies considered it (Akbarzadeh *et al.,* 2007; İşbilen *et al.,* 2007); the bodyweight accepted by the normal male rats feed with beverage kefir were much higher than the treated diabetic ones.

After calculated the total body weight gain and the percentage, it explained as well the increase of the total BW gain and its percentage in the normal males treated with kefir more than the diabetic group which taking kefir.Some researchers showed the potential effects of the components of dairy products like kefir for decreasing the body weight (Zemel *et al.,* 2000; Zemel, 2003; Zemel *et al.,* 2004; Zemel *et al.,* 2005; Teegarden, 2005; Mirmiran *et al.,* 2005; Major *et al.,* 2007; Shahar *et al.,* 2007; Vergnaud *et al.,* 2008; Van Loan, 2009; Sanders, 2012).A diet rich in dairy calcium intake enhances weight reduction in type 2 diabetic patients (Danit *et al.,* 2007).Akbarzadeh. *et al.,* (2007) explained that the bodyweight of the diabetic rats

which induced experimentally by streptozotocin decreased comparing with the normal rats.Yasamin *et al.,* (2016) observed in their study that Kefir drink leads to a similar weight loss, compared with milk in overweight or obese premenopausal women.A total of 13 studies revealed the effects of probiotic supplementation consumption and found that the administration of probiotics was associated with a significantly larger reduction in BMI and weight loss (Borgeraas *et al.,* 2018).Zemel *et al.,* (2004) hypothesized that a dairy-rich diet containing kefir drink would lead to a greater weight loss, as kefir drink might have the antiobesity properties of dairy products and probiotics in combination.Chen *et al.,* (2012) did not support the beneficial effects of increasing dairy consumption on body weight and fat loss in studies without energy restriction.Previous reviews have found probiotics to reduce body weight in adults, and the reported effect level was small (Zhang *et al.,* 2015; Dror *et al.,* 2017).

Other studies found probiotics to increase weight in infants and children while having the opposite effect among adults (Dror *et al.,* 2017).A different study observed that Kefir's treatment resulted in a significant increase in body mass gain (Fabiane *et al.,* 2016).

Majority of randomized controlled trials (RCTs) have failed to show the potential weight-reducing effects of dairy products in the absence of energy restriction (Baran *et al.,* 1990; Zemel *et al.,* 2005; Palacios *et al.,* 2011; Van Meijl and Mensink, 2010; Chen *et al.,* 2012).

However, some studies observed that rat body weight gain was similar in both groups (control and kefir); No significant differences were found (Elena *et al.,* 2007; Sahin and Yardimci, 2009; Ataşoğlu *et al.,* 2010; Kızak and Çelik, 2012; Aliakbarpour *et al.,* 2013; Salaj *et al.,* 2013; Piccolo *et al.,* 2015).

Oral administration of viable strains of bacteria (probiotics) has been proposed as a way of enhancement the gut ecosystem to favour weight reduction or decrease weight gain; however, the mechanisms may influence gut microbiota are largely unknown (Sanders, 2016).In the meantime, several recent studies have, however, found probiotic supplementation to promote both weight gain and weight loss (Zhang *et al.,* 2015; Drissi *et al.,* 2016). Probiotics which provided in the form of fermented dairy products had a highly growing evidence regarding the significant contribution of gut microbiota to energy homeostasis and weight control (Arora and Sharma, 2011).

We showed that the mean length of the abdominal circumference which measured during the sacrifice process was nearly in the same range for all the rat males groups, there were no differences between the normal and diabetic ones; by another way no significance in the waist circumference (WC) in the untreated and treated groups.

Some studies involving adults have shown negative relationships between dairy intake and a variety of anthropometric indicators for general and/or central obesity; which were suggestive of inverse associations of total dairy intake with weight and WC (Wang *et al.,* 2014).

In the other study, receiving an adequate-dairy in overweight or obese adults significantly reduced WC and body fat but had no effect on body weight (Stancliffe *et al.,* 2011; Chang *et al.,* 2011; Kadooka *et al.,* 2013).

REFERENCES

Agerholm-Larsen, L.; Raben, A.; Haulrik, N.; Hansen, A. S.; Manders, M. and Astrup, A. (2000): Effect of 8-week intake of probiotic milk products on risk factors for cardiovascular diseases. Eur. J. Clin. Nutr.; 54(4):288–297.

Akbarzadeh, A.; Norouzian, D.; Mehrabi, M. R.; Jamshidi, S. h.; Farhangi, A.; Allah Verdi, A.; Mofidian, S. M. A. and Lame Rad, B. (2007): Induction of diabetes by streptozotocin in rats. Ind. J. of Clin. Bioch.; 22(2)60-64.

Aliakbarpour, H. R.; Chamani, M.; Rahimi, G.; Sadeghi, A. A. and Qujeq, D. (2013): Intermittent feeding programme and

addition of Bacillus subtilis based probiotics to the diet of growing broiler chickens: influence on growth, hepatic enzymes and serum lipid metabolites profile. Tierzucht.; 56(40): 410-422.

Arora, T. and Sharma, R. (2011): Fermentation potential of the gut microbiome: implications for energy homeostasis and weight management. Nutr. Rev.; 69(2):99–106.

Ataşoğlu, C.; Akbağ, H. I.; Tölü, C.; Daş, G.; Savaş, T. and Yurtman, İ. Y. (2010): Effects of kefir as a probiotic source on the performance of goat kids. South Afric. J. of Animal Sci.; 40(4):363-370.

Baothman, O. A.; Zamzami, M. A.; Taher, I.; Abubaker, J. and Abu-Farha, M. (2016): The role of gut microbiota in the development of obesity and diabetes. Lipids in Health and Disease; 15(108):1-8.

Baran, D.; Sorensen, A.; Grimes, J.; Lew, R.; Karellas, A.; Johnson, B. and Roche, J. (1990): Dietary modification with dairy products for preventing vertebral bone loss in premenopausal women: a three year prospective study. J. Clin. Endocrinol. Metab.; 70(1):264–270.

Borgeraas, H. L.; Johnson, K.; Skattebu, J.; Hertel, J. K. and Hjelmesaeth, J. (2018): Effects of probiotics on body weight, body mass index, fat mass and fat percentage in subjects with overweight or obesity: a systematic review and meta-analysis of randomized controlled trials. Etiol. and Pathophysiol.; 19(2):219-232.

Bos, M. and Agyemang, C. (2013): Prevalence and complications of diabetes mellitus in Northern Africa, a systematic review. BMC Public Health; 13 (387):1-7.

Chang, B. J.; Park, S.U.; Jang, Y.S.; Ko, S. H.; Joo, N. M.; Kim, S. I.; Kim, C. H. and Chang, D. K. (2011): Effect of functional yogurt NY-YP901 in improving the trait of metabolic syndrome. Eur. J. Clin. Nutr.; 65(11):1250–1255.

Chen, M.; Pan, A.; Malik. V. S. and Hu, F. B. (2012): Effects of dairy intake on body weight and fat: a meta-analysis of randomized controlled trials. Am. J. Clin. Nutr.; 96(4):735–747.

Danit, R. S.; Relly, A.; Asher, E.; Hillel, V. and Drora, F. (2007): Does Dairy Calcium Intake Enhance Weight Loss Among Overweight Diabetic Patients? Diabetes Care; 30(3):485–489.

Davis, C. D. (2016): The gut microbiome and its role in obesity. Nutr. Today; 51(4):167–174.

Drissi, F.; Raoult, D. and Merhej, V. (2016): Metabolic role of lactobacilli in weight modification in humans and animals. Microb. Pathog.; 106:182–194.

Dror, T.; Dickstein, Y.; Dubourg, G. and Paul, M. (2017): Microbiota manipulation for weight change. Microb. Pathog; 106:146–161.

Ejtahed, H. S.; Soroush, A. R.; Angoorani, P.; Larijani, B. and Hasani-Ranjbar, S. (2016): Gut microbiota as a target in the pathogenesis of metabolic disorders: A new approach to novel therapeutic agents. Horm. Metab. Res.; 48(06): 349–358.

Elena, U.; Jaione, B.; Patricia, A.; Aurora, I.; Florencio, M. And Francisco, C. I. (2007): Intestinal beneficial effects of kefir-supplemented diet in rats. Nutr. Res.; 27(10): 653–658.

Fabiane, R. M.; Giovana, R. P.; Adelson, M. R.; Cristina, S. B. B.; Marcelo, M. R.; Marice, N. O.; Margaret, G. M. and Elisa, M. S. H. (2016): Immunomodulation and nitric oxide restoration by a probiotic and its activity in gut and peritoneal macrophages in diabetic rats. Clin.Nutr.; 35(5):1066-1072.

Giovana, R. P. A.; Fabiane, R. M. A.; Adelson, M. R. A.; Marcelo, M. R. C.; Cristina, S. B. B. D.; Marice, N. O. D.; Silvia, S. M. I. B.; Sergio, R. R. A. b.; Talita, R. C. S. E.; Lucia, C. A. E. and Elisa, M. S. H. (2014): Kefir administration reduced progression of renal injury in STZ-diabetic rats by lowering oxidative stress. Nitric. Oxide.; 37(15):53–60.

Guarner, F.; Perdigon, G.; Corthier, G. r.; Salminen, S.; Koletzko, B. and Morelli, L. (2005): Should yoghurt cultures be considered probiotic? Br. J. Nutr.; 93(6):783-786.

Guven, A. and Gulmez, M. (2003): The effect

of kefir on the activities of GSH-Px, GST, CAT, GSH and LPO levels in carbon tetrachloride-induced mice tissues. J. Vet. Med. B Infect.; 50(8):412–416.

Hadisaputro, S.; Djokomoeljanto, R. R.; and Judiono Soesatyo, M. H. (2012): The effects of oral plain kefir supplementation on proinflammatory cytokine properties of the hyperglycemia Wistar rats induced by streptozotocin. Acta Med. Indones; 44(2):100–104.

İşbilen, B.; Arı, Z.; Var, O.; Onur, E. and Uyanık, B. S. (2007): The Effect of DHEAS on Leptin, Lipid Profile, and Endothelial Function in the Rats Exposured To High-Fat Diet. FÜ Sağ Bil Derg.; 21(3):109-116.

Judiono; Djokomoeljanto and dan Hadisaputro, S. (2011): Effects of oral clear kefir probiotics on glycemic status, lipid peroxidation, antioxidative properties of streptozotocin induced hyperglycemia wistar rats. Gizi Indon.; 34(1):1-6.

Kadooka, Y.; Sato, M.; Ogawa, A.; Miyoshi, M.; Uenishi, H.; Ogawa, H.; Ikuyama, K.; Kagoshima, M. and Tsuchida, T. (2013): Effect of Lactobacillus gasseri SBT2055 in fermented milk on abdominal adiposity in adults in a randomised controlled trial. Br J. Nutr.; 110(9):1696–1703.

Kızak, M. K. and Çelik, H. T. (2012): The effects of different dosage of kefir with different durations on growth performances and antioxidant system in the blood and liver tissues of Çoruh trout (Salmo coruhensis). Turkish J.of Fish. and Aquat. Sci.; 12(2):277-283.

Kwon, O. K.; Ahn, K. S.; Lee, M. Y.; Kim, S. Y.; Park, B. Y.; Kim, M. K.; Lee, I. Y.; Oh, S. R. and Lee, H. K. (2008): Inhibitory effect of kefiran on ovalbumin-induced lung inflammation in a murine model of asthma. Arch. Pharm Res.; 31(12):1590–1596.

Lee, S. J.; Bose, S.; Seo, J. G.; Chung, W. S.; Lim, C. Y. and Kim, H. (2014): The effects of co-administration of probiotics with herbal medicine on obesity, metabolic endotoxemia and dysbiosis: a randomized double-blind controlled clinical trial. Clin. Nutr.; 33(6): 973–981.

Major, G. C.; Alarie, F.; Dore, J.; Phouttama, S. and Tremblay, A. (2007): Supplementation with calcium + vitamin D enhances the beneficial effect of weight loss on plasma lipid and lipoprotein concentrations. Am. J. Clin. Nutr.; 85(1):54–59.

Mirmiran, P.; Esmaillzadeh, A. and Azizi, F. (2005): Dairy consumption and body mass index: an inverse relationship. Int. J. Obes.; 29(1):115–121.

Palacios, C.; Bertran J. J.; Rios, R.E. and Soltero, S. (2011): No effects of low and high consumption of dairy products and calcium supplements on body composition and serum lipids in Puerto Rican obese adults. Nutrition; 27(5):520–525.

Piccolo, G.; Bovera, F.; Lombardi, P.; Mastellone, V.; Nizza, S.; Di Meo, C. and Nizza, A. (2015): Effect of Lactobacillus plantarum on growth performance and hematological traits of European sea bass (Dicentrarchus labrax). Aquac. Intern.; 23(4):1025-1032.

Sahin, E. H. and Yardimci, M. (2009): Effects of Kefir as a Probiotic on Growth Performance and. J. of Ani. and Veter. Adv.; 8(3):562-567.

Salaj, R.; Štofilová, J.; Šoltesová, A.; Hertelyová, Z.; Hijová, E.; Bertková, I. and Bomba, A. (2013): The effects of two Lactobacillus plantarum strains on rat lipid metabolism receiving a high fat diet. The Scientific World Journal; 2013(135142):1-7.

Sanders, T. A. (2012): Role of dairy foods in weight management. Am. J. Clin. Nutr.; 96(4):687–688.

Sanders, M. E. (2016): Probiotics and microbiota composition. BMC Med.; 14(1): 82-84.

Shabana, Shahid, S. U. and Irfan, U. (2018): The gut microbiota and its potential role in obesity. Fut. Microb.; 13(5):589–603.

Shahar, D. R.; Abel, R.; Elhayany, A.; Vardi, H. and Fraser, D. (2007): Does dairy calcium intake enhance weight loss among overweight diabetic patients? Diabetes Care; 30(3):485–489.

Sherif, S. and Bauer E, S. (2015): Economic

development and diabetes prevalence in MENA countries: Egypt and Saudi Arabia comparison. World J. Diabetes; 6(2):304–311.

Srinivasan, K.; Patole, P. S.; Kaul, C. L. and Ramarao, P. (2004): Reversal of glucose intolerance by pioglitazone in high fat diet-fed rats. Methods Find Exp. Clin. Pharmacol.; 26(5):327-333.

Stancliffe, R. A.; Thorpe, T. and Zemel, M. B. (2011): Dairy attenuates oxidative and inflammatory stress in metabolic syndrome. Am. J. Clin. Nutr.; 94(2):422–430.

Teegarden, D. (2005): The Influence of Dairy Product Consumption on Body Composition. The J. of Nutr.; 135(12):2749–2752.

Van Loan, M. (2009): The role of dairy foods and dietary calcium in weight Management. J. of the Am. College of Nutr.; 28(1):120-129.

Van Meijl, L. E. and Mensink, R. P. (2010): Effects of low-fat dairy consumption on markers of low-grade systemic inflammation and endothelial function in overweight and obese subjects: an intervention study. Br. J. Nutr.; 104(10):1523–1527.

Vergnaud, A. C.; Peneau, S.; Chat-Yung, S.; Kesse, E.; Czernichow, S.; Galan, P.; Hercberg, S. and Bertrais, S. (2008): Dairy consumption and 6-y changes in body weight and waist circumference in middle-aged French adults. Am. J. Clin. Nutr.; 88(5):1248–1255.

Wang, H.; Troy, L. M.; Rogers, G. T.; Fox, C. S.; McKeown, N. M.; Meigs, J. B. and Jacques, P. F. (2014): Longitudinal association between dairy consumption and changes of body weight and waist circumference: the Framingham Heart Study. Int. J. Obes.; 38(2):299–305.

Yadav, H.; Jain, S. and Sinha, P. R. (2008): Oral administration of dahi containing probiotic Lactobacillus acidophilus and Lactobacillus casei delayed the progression of streptozotocin-induced diabetes in rats. J. Dairy Res.; 75(2):189–195.

Yasamin, F.; Shiva, F.; Mohammad, J. Z. and Sayed, H. R. T. (2016): Kefir drink leads to a similar weight loss, compared with milk, in a dairy-rich non-energy-restricted diet in overweight or obese premenopausal women: a randomized controlled trial. Eur. J. Nutr.; 55(1):295–304.

Zemel, M. B.; Shi, H.; Greer, B.; Dirienzo, D. and Zemel, P. C. (2000): Regulation of adiposity by dietary calcium. FASEB J.; 14(9):1132–1138.

Zemel, M. B.; Thompson, W.; Milstead, A. and Morris, K. Campbell P. (2004): Calcium and dairy acceleration of weight and fat loss during energy restriction in obese adults. Obes Res.; 12(4):582–590.

Zemel, M.B.; Richards, J.; Mathis, S.; Milstead, A.; Gebhardt, L. and Silva, E. (2005): Dairy augmentation of total and central fat loss in obese subjects. Int. J. Obes.; 29(4):391–397.

Zemel, M. B. (2003): Role of dietary calcium and dairy products in modulating adiposity. Lipids; 38(2):139–146.

Zhang, Q.; Wu, Y. and Fei, X. (2015): Effect of probiotics on body weight and body-mass index: a systematic review and meta-analysis of randomized, controlled trials. Int. J. Food Sci. Nutr.; 67(5):571–580.

Ziegler, A. G.; Rewers, M.; Simell, O. et al. (2013): Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. JAMA; 309(23): 2473–2479.