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Effects of Probiotics on Biomarkers of Oxidative Stress and Inflammatory Factors in Petrochemical Workers: A Randomized, Double-blind, Placebo-controlled Trial

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Abstract

Background:

The aim of the current study was to determine effects of probiotic yoghurt and multispecies probiotic capsule supplementation on biomarkers of oxidative stress and inflammatory factors in petrochemical workers.

Methods:

This randomized, double-blind, placebo-controlled trial was done among petrochemical workers. Subjects were randomly divided into three groups to receive 100 g/day probiotic yogurt (n = 12) or one probiotic capsule daily (n = 13) or 100 g/day conventional yogurt (n = 10) for 6 weeks. The probiotic yoghurt was containing two strains of *Lactobacillus acidophilus* and *Bifidobacterium lactis* with a total of min 1×10^7 CFU. Multispecies probiotic capsule contains seven probiotic bacteria spices *Actobacillus casei* 3×10^3, *L. acidophilus* 3×10^7, *Lactobacillus rhamnosus* 7×10^9, *Lactobacillus bulgaricus* 5×10^8, *Bifidobacterium breve* 2×10^{10}, *Bifidobacterium longum* 1×10^9 and *Streptococcus thermophilus* 3×10^8 CFU/g. Fasting blood samples were obtained at the beginning and end of the trial to quantify biomarkers of oxidative stress and inflammatory factors.

Results:

Although a significant within-group decrease in plasma protein carbonyl levels was seen in the probiotic capsule group (326.0 ± 308.9 vs. 251.0 ± 176.3 ng/mL, P = 0.02), the changes were similar among the three groups. In addition, significant within-group decreases in plasma iso prostaglandin were observed in the probiotic supplements group (111.9 ± 85.4 vs. 88.0 ± 71.0 pg/mL, P = 0.003) and in the probiotic yogurt group (116.3 ± 93.0 vs. 92.0 ± 66.0 pg/mL, P = 0.02), nevertheless there were no significant change among the three groups.

Conclusions:

Taken together, consumption of probiotic yogurt or multispecies probiotic capsule had beneficial effects on biomarkers of oxidative stress in petrochemical workers.

Keywords: Iso prostaglandin, oxidative stress, petrochemical workers, probiotics, protein carbonyl

INTRODUCTION

Oxidative stress which is referred to imbalance between prooxidants and antioxidants,[1] is very common in petrochemical workers. Exposure to petrochemical contaminants such as benzene, toluene, ethylbenzene, and xylenes and diesel exhaust particulate leads to increased production of reactive oxygen species (ROS) and free radicals, which in turn would result in increased oxidative stress.[2,3,4] Increase in oxidative stress is widely thought as a mechanism involved in developing cancer, diabetes, Parkinson's disease, Alzheimer's disease, cardiac disease, neurological and psychiatric disorders.[5] Moreover, increased risk of some cancers such as melanoma, bladder and prostate carcinoma and pulmonary mesothelioma has been reported in petrochemical workers.[6]

There are several strategies to reduce oxidative stress including use of antioxidants especially vitamins A, C, and E,[7] carotenoids,[8] polyphenols[9] as well as diets rich in vegetables and fruits.[10] Recently, the use of probiotics to reduce the oxidative stress has been proposed. Probiotics are microorganisms that are believed to provide health benefits for humans and animals when consumed.[11] To the best of knowledge of the authors, no studies are available indicating the effects of probiotics on biomarkers of oxidative stress and inflammatory factors among petrochemical workers. However, it has been assessed on these markers in pregnant women[12] and in subjects with type 2 diabetes mellitus.[13] Asemi *et al.*[14] have carried out a study on pregnant women and have observed a significant decrease of some biomarkers of oxidative stress following use of probiotic yoghurt containing *Lactobacillus acidophilus* La5 and *Bifidobacterium animalis* for 9 weeks. In addition, use of probiotic milk containing *Lactobacillus fermentum* ME-3 for 3 weeks, reduced some oxidative stress biomarkers.[15] However, Meyer *et al.*,[16] did not report such beneficial effects.

Probiotics may reduce oxidative stress through affecting cytokine production, decreasing interleukin 1, tumor necrosis factor-alpha[17] and increasing glutathione (GSH) levels.[14] In addition, blocking superoxide production and hydroxyl radicals,[18] decrease of prooxidants levels,[19] stimulation and reinforcement of immune system[20] may be explained further favorable effects by probiotics on oxidative stress. We hypothesized that probiotic supplementation might positively affect biomarkers oxidative stress and inflammatory factors in petrochemical workers. As to our knowledge no previous study has evaluated effects of probiotics on biomarkers oxidative stress and inflammatory factors in petrochemical workers, the aim of our study was to determine effects of probiotic yoghurt and multispecies probiotic supplements on biomarkers oxidative stress and inflammatory factors in petrochemical workers.

METHODS

Participants

This randomized, double-blind, placebo-controlled trial was performed in Tehran, Iran, during February 2014 to March 2014. The current study was approved by the ethical committee of research deputy of Tehran University of Medical Sciences. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki's Declaration of 1975, as revised in 2008. In addition, we obtained written informed consent from all subjects.

The trial was registered in the Iranian website (www.irct.ir) for registration of clinical trials (IRCT code: IRCT201406222394N11). Petrochemical workers and aged 20–60 years old were included in this study. Individuals were excluded if they were using insulin or vitamin supplements, or had chronic kidney disease, lung and chronic or acute inflammatory disease, hepatic or thyroid diseases, severe intestinal disease, peptic ulcer, pregnant, allergies or the use of antibiotics and anti-inflammatory drugs. Based on the suggested formula for parallel clinical trials, considering the type one error of 5% and the study power of 80%, we reached the sample size of 10 for each group. However, we recruited 13 persons in each group (totally, 39 persons) to compensate for the probable loss to follow-up.

Study design

To obtain detailed information about the dietary intakes of study participants, all workers entered into a 2-week run-in period; during which all persons had to refrain from taking any other probiotic or synbiotic food. During the run-in period after stratification for body mass index (BMI) (<30 and ≥30 kg/m^2) and age (<40 and ≥40 year), participants were requested to record their dietary intakes for three nonconsecutive days. At the end of run-in period, subjects were randomly divided into three groups to receive 100 g/day probiotic yogurt + one placebo capsule (n = 12) or one probiotic capsule daily +100 g/day conventional yogurt (n = 13) or 100 g/day conventional yogurt + one placebo capsule (n = 10) for 6 weeks. Randomization and allocation were concealed from the researcher and participants until after the main analyses had been completed. A trained nutritionist at petrochemical technology company did the randomized allocation sequence, enrolled participants and assigned participants to interventions. In during intervention, subjects were asked not to change their routine physical activity or usual diets and not to consume any probiotic products other than the one provided to them by the investigators. In addition, they were asked to avoid consuming any fermented and synbiotic products. Probiotic and conventional yogurts or multispecies probiotic supplements were provided for participants every day. All participants provided three dietary records (1 weekend day and 2 weekdays) throughout the intervention and three physical activity records to make sure that they maintained their usual diet and physical activity during the study. Both dietary and physical activity records were taken at week 1, 3 and 5 of the intervention. The dietary records were based on estimated values in household measurements. To obtain nutrient intakes of participants based on these 3-day food diaries, we used Nutritionist IV Software (First Databank, San Bruno, CA, USA) modified for Iranian foods.

Assessment of variables

Weight was measured at the beginning study and 6 weeks after the intervention at petrochemical technology company, Tehran, Iran by trained nutritionist. Body weight was measured in an overnight fasting status without shoes in a minimal clothing state by the use of a digital scale (Seca, Hamburg, Germany) to the nearest 0.1 kg. Height was measured using a nonstretched tape measure (Seca, Hamburg, Germany) to the nearest 0.1 cm. BMI was calculated using the height and weight measurements (weight in kg/[height in meters]^2). Fasting blood samples (10 mL) were obtained at the beginning study and end-of-trial at Kavosh medical laboratory in an early morning after an overnight fast. Blood was collected in 2 separate tubes: (1) One without EDTA to separate the serum, in order to quantify serum 8-oxo-7, 8-dihydroguanine (8-oxo-G), high sensitivity C-reactive protein (hs-CRP) and interferon gamma (INF-γ) levels and (2) another one containing EDTA to examine protein carbonyl (PC) and prostaglandin F2-alpha (8-iso-PGF2a). Blood samples were immediately centrifuged (Universal, Germany) at 3500 rpm for 10 min to separate serum. Then, the samples were stored at −80°C until analysis at the Kavosh medical laboratory.

Outcomes

In the present study, the primary outcomes were biomarkers of oxidative stress including PC, 8-iso-PGF2a and 8-oxo-G. Plasma PC (Hangzhou Eastbiopharm, China) with intra- and inter-assay coefficient of variations (CVs) <10%, 8-iso-PGF2a (Hangzhou Eastbiopharm, China) with intra- and inter-assay CVs 12% and 8-oxo-G levels (Hangzhou Eastbiopharm, China) with intra- and inter-assay CVs of 10% and 12% were quantified with ELISA methods. In our study, the secondary outcomes were inflammatory factors. Serum hs-CRP was quantified using ELISA kit (LDN, Nordhorn, Germany) with intra- and inter-assay CVs of 2.2% and 4.5%, respectively. Serum INF-γ was determined using ELISA kit (Boster, USA) with intra- and inter-assay CVs of 5.5 and 7.8%, respectively.

Characteristics of yogurts and supplements

The probiotic yoghurt was containing two strains of *L. acidophilus* LA5 and *Bifidobacterium lactis* BB12 with a total of min 1×10^7 CFU. The conventional yoghurt contained the starter cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Both yogurts' PH was in the range of 4.3–4.5 and their fat content was 2.5%. Multispecies probiotic capsule contains seven probiotic bacteria spices *Actobacillus casei* 3×10^3, *L. acidophilus* 3×10^7, *Lactobacillus rhamnosus* 7×10^9, *L. bulgaricus* 5×10^8, *Bifidobacterium breve* 2×10^{10}, *Bifidobacterium longum* 1×10^9, *S. thermophilus* 3×10^8 CFU/g and 100 mg fructo-oligosaccharide with lactose as carrier substances. It is well known that it would be more appropriate if the strains used for human consumption originated from the human intestinal tract, well-characterized, able to survive the rigors of the digestive tract and possibly colonize, biologically active against the target as well as to be stable and amenable to commercial production and distribution.[21] Placebo (the same substance without bacteria) was packed in identical capsules and coded by the producer to guarantee blinding. In every production, the content of the yogurt bacteria were measured routinely, and the survival of the probiotic bacteria was also measured in the day 1, 3, and 5. The probiotic bacteria in yogurt and capsule were alive till the end of shelf life (day 5 for yogurt and 6 months for capsule). Yogurts were provided by Pegah Company, Tehran, Iran. The multispecies probiotic and placebo capsules were provided by ZistTakhmir Co., Tehran, Iran.

Statistical methods

The normality of the variables was examined by the Kolmogorov–Smirnov test. Log transformation was performed for nonnormally distributed variables. To detect differences in general characteristics, dietary intakes and to determine the effect of probiotic yogurt, probiotic capsule and conventional yogurt on biomarkers of oxidative stress and inflammatory factors among the three groups, one-way analysis of variance was used for normally distributed variables and Wilcoxon signed ranks test was used for nonnormally distributed variables. To identify within group differences (baseline and end-of-trial), we used paired-samples *t*-test for normally distributed variables and Kruskal–Wallis test for nonnormally distributed variables. The changes across three groups were compared using Bonferoni *post-hoc* pair-wise comparisons. P values were considered statistically significant at $P < 0.05$. The statistical analyses were carried out using the statistical packages for SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Among subjects in the placebo group, three persons (withdrawn [n = 3]) were excluded. The exclusions in the probiotic yogurt group was 1 individual (withdrawn [n = 1]). Finally, 35 participants (placebo group [n = 10], probiotic yogurt group [n = 12] and multispecies probiotic supplements [n = 13]) completed the trial [Figure 1].

No serious adverse reactions were reported following the administration of probiotic yogurt and multispecies probiotic supplements in petrochemical workers throughout the study. Mean age, height, weight and BMI at baseline and 6 weeks after the intervention were not significantly different among the three groups [Table 1].

At the study beginning, no significant differences were found among the three groups in terms of dietary intakes. Comparing the dietary intakes in run-in period and throughout the study separately in each group, we observed no significant within-group differences in dietary intakes of energy, fat, saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, cholesterol, dietary fiber, vitamins E, C, zinc, magnesium, manganese and selenium (data not shown).

After 6 weeks of intervention, although a significant within-group decrease in plasma PC levels was seen in the probiotic capsule group (326.0 ± 308.9 vs. 251.0 ± 176.3 ng/mL, $P = 0.02$), the changes were similar among the three groups [Table 2]. In addition, significant within-group decreases in plasma iso prostaglandin levels were observed in the probiotic supplements group (111.9 ± 85.4 vs. 88.0 ± 71.0 pg/mL, $P = 0.003$) and in the probiotic yogurt group (116.3 ± 93.0 vs. 92.0 ± 66.0 pg/mL, $P = 0.02$), nevertheless there are no significant change among the three groups. We did observe no significant effect of probiotic yoghurt consumption and multispecies probiotic capsule supplementation on 8-dihydroguanine and inflammatory factors.

DISCUSSION

Our study assessed the effects of probiotic yoghurt and multispecies probiotic capsule supplementation on biomarkers of oxidative stress and inflammatory factors in petrochemical workers for 6 weeks. The major finding was that probiotic yoghurt and multispecies probiotic capsule supplementation had favorable effects on the biomarkers of oxidative stress in petrochemical workers. To the best of our knowledge, this study is the first that reports the effect of probiotic yoghurt and multispecies probiotic capsule supplementation on biomarkers of oxidative stress and inflammatory factors in petrochemical workers. Petrochemical workers are susceptible to increase in oxidative stress.[5] The current study demonstrated that consumption of probiotic capsule has resulted in significant decreases in plasma PC and iso prostaglandin levels as well as taking probiotic yogurt led to a significant reduction in plasma PC levels among petrochemical workers for 6 weeks, but did not affect serum 8-dihydroguanine levels. In consistent with our study, Lamprecht *et al.*[22] showed that probiotic supplementation for 14 weeks decreased PC levels among athletes, but it was not statistically significant. In addition, *B. breve* attenuated oxidative stress in skin via preventing ROS generation and suppressing oxidation of protein and lipids and xanthine oxidase activity.[23] The same as findings was also observed following the administration of *B. longum* during industrial process[24] and taking multispecies probiotic capsule among diabetic patients.[25] In another study, Asemi *et al.*[14] demonstrated a significant reduction in dihydroguanine levels after intake of probiotic yogurt containing *L. acidophilus* and *B. lactis* among pregnant women after 9 weeks. However, some researchers could not observe such beneficial effects of probiotic supplementation on the biomarkers of oxidative. For instance, taking a probiotic preparation (VSL#3) for 7 days could not affect biomarkers of oxidative stress among critically ill patients.[26] In addition, received multi-species probiotics supplements (10^{10} CFU/day) for 14 weeks did not affect malondialdehyde levels among men.[22] The beneficial effects of intake of probiotics on biomarkers of oxidative stress might be attributed to its impact on decreased lipid peroxidation such as oxidized low-density lipoprotein, 8-isoprostanes and GSH redox ratio[15] and increased GSH levels in skin and serum through augmentation of glutamate-cysteine-ligase activity.[27]

Our study revealed that intake of probiotic yogurt and probiotic capsule did not influence inflammatory factors among petrochemical workers for 6 weeks. Previous studies, the beneficial effects of probiotics on inflammatory factors have reported among immune-compromised patients[28] and patients with rheumatoid arthritis.[29] In line with our study, administration of two Lactobacillus strains, one Bifidobacterium strain, one Propionibacterium strain plus galacto-oligosaccharides did not affect serum CRP levels among men with a low serum enterolactone concentration after 6 weeks.[30] In addition, taking 1500 mg probiotic capsules containing *L. acidophilus*, *L. bulgaricus*, *Lactobacillus bifidum* and *Lactobacillus casei* twice daily was shown no significant changes in inflammatory factors among diabetic patients after 6 weeks.[31] However, the consumption of probiotic yoghurt containing *L. acidophilus* and *Bifidobacterium animalis* among pregnant women after 9 weeks[32] and administration of Bifidobacterium supplements among patients undergoing resection for colorectal cancer[33] has resulted in decreased inflammatory markers. The absent effect of probiotic consumption on inflammatory factors levels in the present study may be result from different study designs, the dosage of used probiotic, the patients under investigation, sample size as well as duration of supplementation.

Some limitations must be taken into account in the interpretation of our findings. Small sample size and short period of supplementation were two main limitations of our study. Long-term interventions might result in greater changes in circulating levels of makers of oxidative stress and inflammation. Furthermore, using other oxidative stress biomarkers including malondialdehyde, GSH reductase, GSH peroxidase and superoxide dismutase could be helpful in the interpretation of results. However, we believed that the assessment of fecal short-chain fatty acids (SCFA) is an appropriate method for compliance, due to budget limitation; we did not assess effects of probiotic on fecal SCFA.

CONCLUSIONS

Taken together, consumption of probiotic yogurt or multispecies probiotic capsule had beneficial effects on biomarkers of oxidative stress in petrochemical workers but did not affect 8-dihydroguanine and inflammatory factors.

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Footnotes

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Conflict of Interest: None declared.

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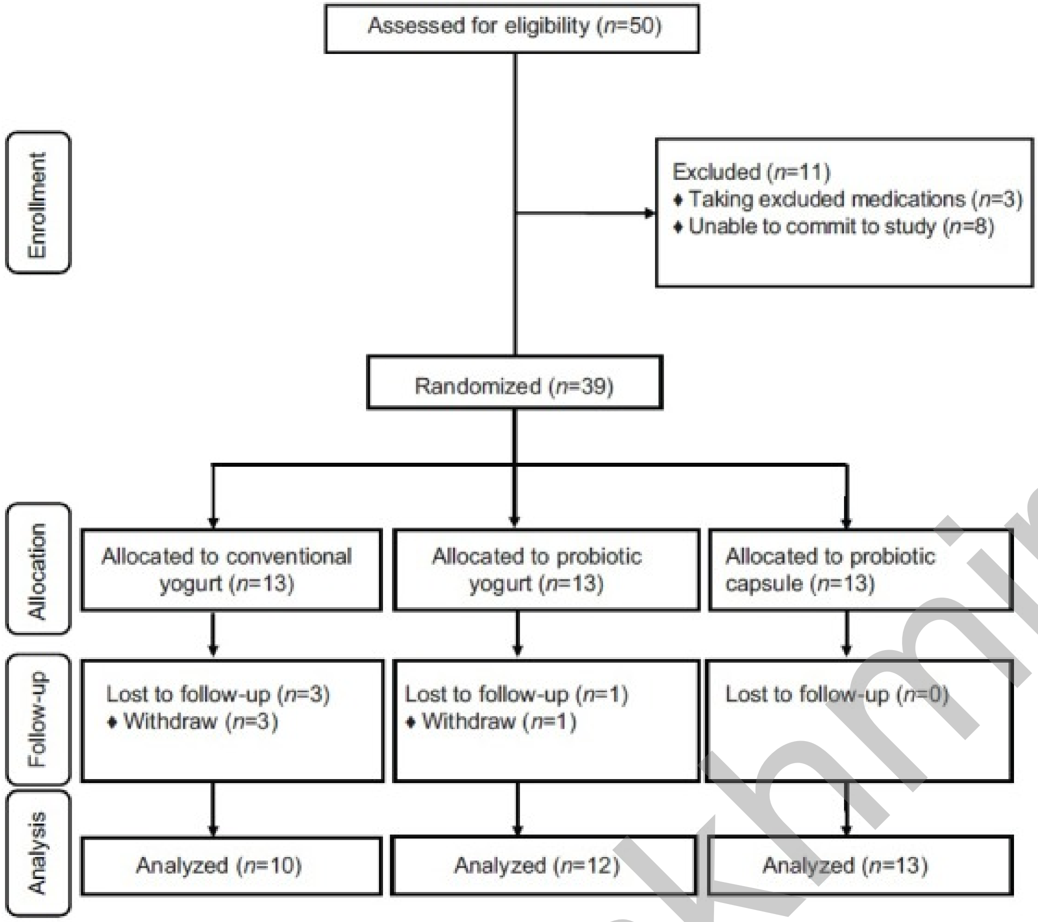
Figures and Tables

Figure 1



Summary of patient flow diagram

Table 1

	Conventional yogurt (n=10)	Probiotic yogurt (n=12)	Probiotic capsule (n=13)	P^b
Age (year)	32.5±4.9	32.8±7.6	32.6±5.5	0.99
Height (cm)	171.9±10.2	170.0±9.5	175.3±0.7	0.39
Weight at study baseline (kg)	75.8±15.0	71.4±16.0	73.2±12.1	0.77
Weight at end-of-trial (kg)	75.4±15.0	70.5±15.6	73.8±11.2	0.70
BMI at study baseline (kg/m^2)	26.0±3.4	23.4±3.5	24.4±3.8	0.27
BMI at end-of-trial (kg/m^2)	25.8±3.4	23.2±3.4	24.7±3.4	0.20

[a]Data are means±SD, [b]Obtained from ANOVA test. SD=Standard deviation, BMI=Body mass index, ANOVA=Analysis of variance

General characteristics of study participants[a]

Table 2

	Conventional yogurt (n=10)			Probiotic yogurt (n=12)			Probiotic capsule (n=13)			P^b
	Week 0	Week 6	Change	Week 0	Week 6	Change	Week 0	Week 6	Change	
Protein carbonyl (ng/mL)	461.8±389.2	450.2±406.2	−11.6±139.9	351.8±352.2	265.3±211.7	−86.4±306.9	326.0±308.9	251.0±176.3*	−75.0±170.8	0.70
Isoprostaglandin (pg/mL)	103.5 (42, 302)	93.5 (20, 253)	−11 (−53, 88)	64.7 (43, 285)	85 (9.9, 214)**	−3.5 (−135, 9)	95 (41, 284)	84 (10, 222)**	−25 (−82, 4)	0.68†
8-oxo-G (ng/mL)	88.3±97.8	84.5±76.3	−3.8±31.3	60.5±69.8	64.0±75.2	3.5±16.9	46.5±52.3	59.1±65.4	12.6±21.2	0.26
hs-CRP (μg/mL)	12.5±11.3	13.5±13.9	1.0±8.7	14.3±10.8	16.0±12.8	1.7±10.0	28.4±50.0	23.9±26.4	−4.4±31.8	0.72
INF-γ (pg/mL)	688.4±394.0	657.9±204.0	−30.5±354.0	610.1±165.2	573.8±109.8	−36.3±180.3	664.9±168.0	638.1±160.2	−26.8±218.4	0.99

*Significant difference with baseline study with pair t-test, **Significant difference with baseline study with Kruskal–Wallis test, †Obtained from Wilcoxon signed ranks test. [a]Values are means±SD for normally distributed variables and median (minimum, maximum) for nonnormally distributed variables, [b]Obtained from ANOVA test. 8-oxo-G=8-oxo-7, 8-dihydroguanine, hs-CRP=High-sensitivity C-reactive protein, INF-γ=Interferon gamma. SD=Standard deviation, ANOVA=Analysis of variance

The effect of daily consumption of probiotic yogurt and probiotic capsule on biomarkers of oxidative stress and inflammatory factors[a]