ORIGINAL ARTICLE

Effect of Different Prebiotics (Galacto oligosaccharide, Fructo oligosaccharide, and Manno Oligosaccharide) on the Inflammatory Markers of High Fat Fed Rats

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ABSTRACT

Background: Obesity is known as a condition of chronic mild inflammation in which there are elevated circulating pro inflammatory cytokines along with alteration of immunity (Lee, 2013). It is associated with the expansion of the adipose tissue. The microbiota of the gut plays a role in obesity and metabolic disorders.
Aim: To compare the effects of three prebiotics, galactooligosaccharides (GOS), fructooligosaccharide (FOS) and mannooligosaccharides (MOS) on the inflammatory markers of high fat fed rats along with a prebiotic.
Study design: Animal experimental study
Place and duration of study The study was carried for 24 weeks in Postgraduate Medical Institute, Lahore.
Methodology: Three weeks of age male Sprague Dawley rats, which were forty in number were distributed among five groups with eight rats in each group : (1) NC (Negative Control) group, fed on standard rat chow (2) PC (Positive control) group (high fat diet i.e., 40% beef tallow in standard rat chow) (3) GOS group (diet supplemented with high fat having 10% GOS) (4) MOS group (diet supplemented with high fat having 10% MOS) (5) FOS group (high fat diet supplemented with 10% FOS).
Results: All the three prebiotics were able to decrease the inflammatory markers significantly but FOS was better in decreasing the CRP and MOS was better in reducing the IL-6 and visceral fat.
Conclusion: Addition of prebiotics cause significant decrease in the inflammatory markers. FOS being most effective in decreasing the CRP and MOS was better in reducing the IL-6 and visceral fat.
Keywords: Prebiotics, Obesity, Inflammatory markers.

INTRODUCTION

Obesity is a condition of chronic mild inflammation in which there is alteration of immunity and raised circulating pro inflammatory cytokines[1]. It is associated with the increase in mass of the adipose tissue due to accumulation of lipid in adipocytes [2]. A variety of proteins and pro inflammatory cytokines are produced by adipose tissue. These include Interleukin -6, Gamma Interferon, Tumor necrotic factor alpha (TNFa), monocyte chemotactic protein I, CRP. Adipose tissue of obese individual produce10 times more IL-6 than lean individuals[3].

C-reactive protein (CRP) has been employed as inflammatory biomarker[4]. The levels of CRP are generally low in healthy patients without acute illness.

Increase risk for cardiovascular disease and insulin resistance are associated with raised levels of CRP[5]. Prebiotics are oligosaccharides that improve the health of host by promoting the survival and activity of the selective bacterial species, which are already present in the colon.[6]. Example of prebiotics includes inulin, fructooligosaccharide and Galactooligosaccharides, mannooligosaccharides.[7]. Prebiotics specifically act on the health-promoting bifidobacteria and lactobacilli[8]. The number of Bifidobacteria is slightly lower in obese adult individuals than in lean people. In type 2 diabetics the number of these bacteria is also decreased compared with non-diabetic patients. It can be concluded from these findings that lower levels of Bifidobacteria contribute in the development of obesity and its related comorbidities[9].

Prebiotics are found naturally in various vegetables, fruits and cereals, in which they function as carbohydrate stores. Examples include, bananas, onion, garlic, wheat and chicory. They are produced on the industrial scale and are easily available in the market[10].

It has been proposed that prebiotics have a role in obesity and other metabolic disorders. In recent years, prebiotics have also been used to improve obesity and its related co-morbiditieis[11,12].

This study was planned to compare the effects of three prebiotics (GOS, FOS and MOS) on inflammatory markers of rats by giving a diet having high fat along with a prebiotic and to see which is better in reducing inflammation.

MATERIALS AND METHODS

This animal experimental study was conducted in PGMI Lahore. A total of forty male Sprague-Dawley rats of 3 weeks age were taken from University Of Animal and Veterinary Sciences Lahore. They were housed in iron cages at 22-24 °C with natural day and night cycle. They were acclimatization for a week with free provision of food and water. They were then divided in five groups.

Group 1 rats were taken as Negative Control (NC), and rats were given standard rat chow diet. Group 2 rats were taken as Positive Control (PC), and rats were given a

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high fat diet (40% beef tallow in normal rat chow).[9] Group 3 (GOS), rats were given a diet supplemented with high fat having 10% GOS. Group 4 (MOS), rats were given a high fat diet supplemented with10% MOS. Group 5 (FOS), rats were given a high fat diet supplemented with 10% FOS.
After the end of 24 weeks blood sample through cardiac puncture was collected from each rat. The serum was separated from the blood by centrifugation at 3500 rpm for 10 min and stored at -20°C. Interleukin -6 and C-reactive protein were measured. Visceral fat was removed immediately after sacrifice and weighed.

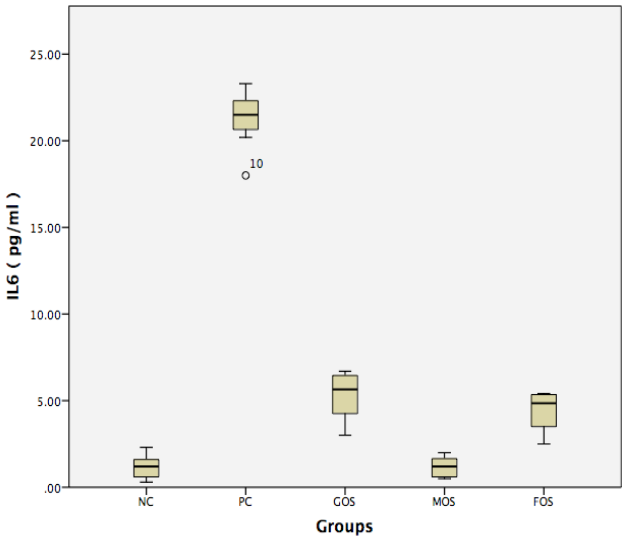
Data was analysed by using IBM-SPSS, version 22. For normally distributed variables One-way ANOVA and Post Hoc Tuckey was applied. For non-normal variables, Kruskal Wallis test and Mann Whitney U test were applied. A *p*-value of < 0.05 was considered statistically significant.

RESULTS

In this study, the effect of three different prebiotics (Galactooligosaccharide, Mannooligosacchaide and Fructoligosaccharide) on Inflammatory markers (IL-6, CRP) was determined.
Interleukin-6: Median (IQR) of NC group was 1.20 pg/ml (0.50—1.60), PC group was 21.50 pg/ml (20.42 —22.45), GOS group was 5.65 pg/ml (3.67—6.52), MOS group was 1.20pg/ml (0.600— 1.72) and FOS group was 4.50 pg/ml (2.75—5.35) as shown in fig-1. Kruskal wallis was applied to see the difference between the mean of all groups which showed a significant p value of (p= 0.018) (Table 1).

Comparisons by Mann Whitney U test revealed that serum interleukin - 6 of PC group was significantly raised when it was compared to the NC group with p = 0.001. Interleukin-6 of treatment groups was also higher as compared to NC group. Difference was also present between NC and GOS (p = 0.001), NC and FOS (p =0.001) but a non significant difference was seen between NC and MOS group (p =0.916) showing that levels of interleukin - 6 in MOS group were closer to NC group.
When compared to PC group, interleukin-6 of all treatment groups were lowered as compare to PC group, multiple comparisons of the three treatment groups revealed a significant difference between PC and GOS (p = 0.001), PC and MOS (p=0.001) PC and FOS (p = 0.001) (Table —1b).
C Reactive Protein (CRP): Median (IQR) of CRP in NC group was 0.60 pg/ml (0.35-1.15), PC group was 12.75 pg/ml (11.02 -15.97), GOS group was 3.15 pg/ml (2.70-3.27), MOS group was 2.05 pg/ml (1.32-3.47) and FOS group was 1.90 pg/ml (0.42-5.47) (Fig- 2). The means of all groups were significantly different when compared by Kruskal Wallis (*p*= 0.000) (Table-2a). Multiple comparisons by Mann Whitney U test revealed that serum C- Reactive Protein levels of PC group was significantly raised as compared to NC group with a *p* value of 0.001. C- Reactive Protein of treatment groups was also higher when compared with NC group. A significant difference was present between NC and GOS (*p*= 0.001), NC and MOS *(p* = 0.005) but the difference was non significant between NC and FOS group (*p* = 0.206) showing that CRP level of FOS group was closer to NC group. When compared to PC group, CRP of all treatment groups were lower in comparison to PC group and multiple comparisons of the three treatment group revealed a significant difference between PC and GOS (*p* = 0.001), PC and MOS (*p* = 0.001) and PC and FOS (*p* = 0.001) (Table-2b).
Visceral Fat Weight: Visceral fat weight of rats in NC group was 8.11±1.80 g, PC group was 13.20±3.91g, GOS was 10.67±1.90 g, MOS group was 8.23±2.05 g and FOS group was 8.88±2.31g (Fig 3). Comparing the means of all groups by ANOVA showed a significant difference of (*p*=0.001) (Table-3a). Comparisons by Post Hoc Tukey Test revealed that the visceral fat weight of PC group was significantly raised than NC group with *p* value 0.003. A non significant difference between NC and GOS, NC and MOS group and NC and FOS group was seen. When compared to PC group, visceral fat weight of all treatment group was lower than the PC group but multiple comparison among three treatment groups revealed a significant difference between PC and MOS groups (*p*=0.003) and PC and FOS group (*p*=0.013). This showed that MOS were able to decrease the visceral fat more than the other two prebiotics (Table-3b).

Figure 1: Serum Interleukin-6 levels {Median (IQR)} of rats in all study groups is an outlier



NC=Negative control, PC= Positive Control, GOS=Galacto oligosaccharide, MOS= Manno oligosaccharide, FOS=Fructo oligosaccharide.

Table-1a: Comparison of Serum Interleukin-6 levels {Median (IQR)} of rats in all study groups by Kruskal Wallis test

Parameter	Group 1 (NC) n =8	Group 2 (PC) n=8	Group 3 (GOS) n=8	Group 4 (MOS) n=8	Group 5 (FOS) n=8	P value
Serum IL-6(pg/ml)	1.20(0.50-1.60)	21.50(20.42-22.45)	5.65(3.67-6.52)	1.20 (0.60-1.72)	4.80 (3.25-5.37)	0.018**

** *p* = 0.01 very significant, NC = Negative control, PC = Positive Control, GOS = Galacto oligosaccharide, MOS = Manno oligosaccharide, FOS = Fructo oligosaccharide.

Table-1b: Comparison of serum Interleukin-6 levels among the groups by Mann Whitney U test

Parameter n = 8	Group	Group	*p* Value	Mean Ranks	
Serum IL – 6 (pg./ml)	NC	PC	0.001***	4.50	12.50
	NC	GOS	0.001***	4.88	12.50
	NC	MOS	0.916	8.38	8.63
	NC	FOS	0.001***	4.50	12.50
	PC	GOS	0.001***	12.50	4.50
	PC	MOS	0.001***	12.50	4.50
	PC	FOS	0.001***	12.50	4.50
	GOS	MOS	0.001***	12.50	4.50
	GOS	FOS	0.051	10.81	6.19
	MOS	FOS	0.001***	4.50	12.50

*** p = 0.00 highly significant, NC = Negative control, PC = Positive Control, GOS = Galacto oligosaccharide, MOS = Manno oligosaccharide, FOS = Fructo oligosaccharide

Figure-2: Serum C- reactive protein {Median (IQR)} of rats in all study groups

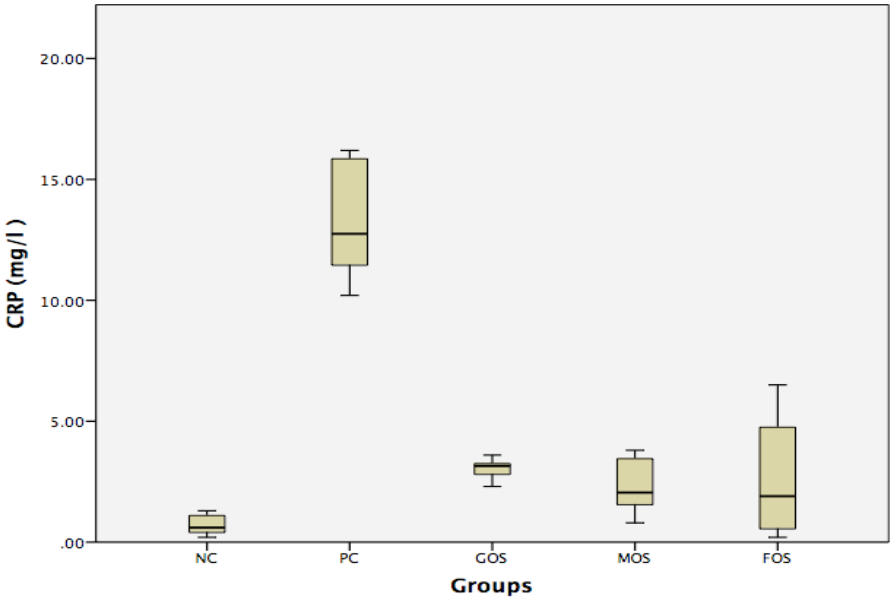


Table-2a: Comparison of serum C- reactive protein levels {Median (IQR)} of rats in all study groups by Kruskal Wallis test

Parameter	Group 1 (NC) n =8	Group 2 (PC) n=8	Group 3 (GOS) n=8	Group 4 (MOS) n=8	Group 5 (FOS) n=8	P value
Serum CRP(pg/ml)	0.60(0.35-1.15)	12.75(11.02-15.97)	3.15(2.70-3.27)	2.05(1.32-3.47)	1.90 (0.42-5.47)	0.000***

*** p = 0.00 highly significant, NC = Negative control, PC = Positive Control, GOS = Galacto oligosaccharide, MOS = Manno oligosaccharide, FOS = Fructo oligosaccharide

Table-2b: Comparison Of serum C- reactive protein levels among the groups by Mann Whitney U Test

Parameter n = 8	Group 1	Group 2	P value	Mean Ranks	
Serum CRP (pg/ml)	NC	PC	0.001***	4.50	12.50
	NC	GOS	0.001***	4.50	12.50
	NC	MOS	0.005***	5.13	11.88
	NC	FOS	0.206	7.00	10.00
	PC	GOS	0.001***	12.50	4.50
	PC	MOS	0.001***	12.50	4.50
	PC	FOS	0.001***	12.50	4.00
	GOS	MOS	0.293	9.75	7.25
	GOS	FOS	0.461	9.38	7.63
	MOS	FOS	0.598	9.13	7.88

*** p = 0.00 highly significance, NC = Negative control, PC = Positive Control, GOS = Galacto oligosaccharide, MOS = Manno oligosaccharide, FOS = Fructo oligosaccharide.

Figure-3: Visceral fat weight (Mean ± SD) of rats in all study groups

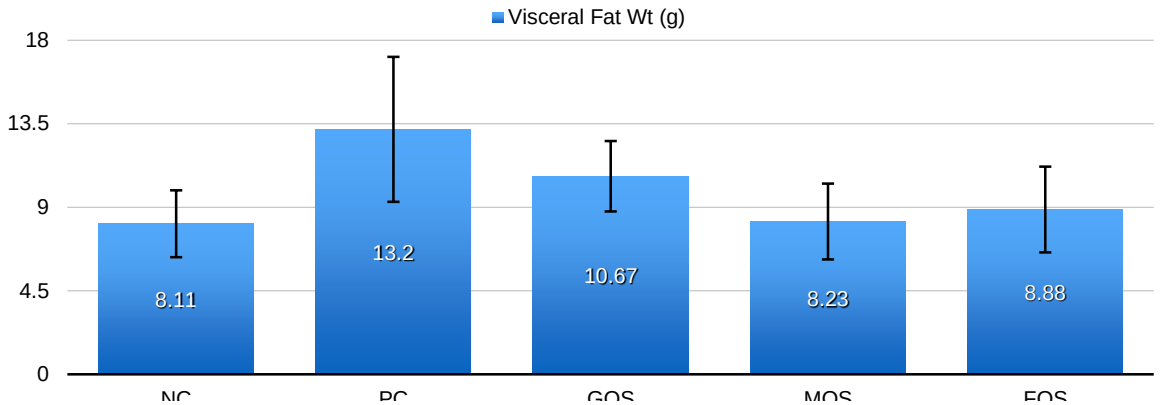


Table-3a: Comparison of visceral fat weight (Mean ± S.D) of rats in all study groups by ANOVA

Parameter	Group 1 (NC) n =8	Group 2 (PC) n=8	Group 3 (GOS) n=8	Group 4 (MOS) n=8	Group 5 (FOS) n=8	P value
Visceral Fat Wt(g)	8.11±1.80	13.20 ±3.91	10.67 ±1.90	8.23 ±2.05	8.88 ±2.31	0.001***

*** *p* = 0.00 highly significant, NC = Negative control, PC = Positive Control, GOS = Galacto oligosaccharide, MOS = Manno oligosaccharide, FOS = Fructo oligosaccharide.

Table-3b: Comparison of visceral fat weight among the groups by Post Hoc Tukey test

Parameter n=8	Group	Group	P value	Mean differences
Visceral fat weight (g)	NC	PC	0.003***	**-5.083**
	NC	GOS	0.276	-2.55
	NC	MOS	1.000	-0.12
	NC	FOS	0.973	-0.77
	PC	GOS	0.287	2.52
	PC	MOS	0.003***	4.96
	PC	FOS	0.013**	4.31
	GOS	MOS	0.321	2.43
	GOS	FOS	0.623	1.78
	MOS	FOS	0.985	-0.65

p = 0.01 very significant, * *p* = 0.00 highly significant
NC = Negative control, PC = Positive Control, GOS = Galacto oligosaccharide, MOS = Manno oligosaccharide, FOS = Fructo oligosaccharide

DISCUSSION

This study was designed to see the effect of prebiotic fibers on the inflammatory markers. The foremost finding is that the visceral fat weight, interleukin -6 and C reactive protein are reduced by prebiotic fibers. Manno oligosaccharide (MOS) is able to decrease the visceral fat weight significantly as compare to the other two prebiotics. Kumao et al. (2006) administered MOS in liquid coffee to healthy adults and concluded that intake of MOS 3g/day decreases fat utilization along with its excess excretion[13].

By supplementation of prebiotics there is a significant decrease in visceral fat. As the visceral fat decreases the inflammatory markers are decreased significantly, indicating a possible link between adipose tissue and secretion of IL-6. IL-6 controls the hepatic synthesis of CRP which can be the reason for higher CRP levels in high fat fed rats and the reductions observed in the groups given prebiotic along with high fat diet. Interleukin - 6 and CRP are lower in three treatment groups as compare to PC group with a significant difference of p = 0.018 and p=0.000 respectively by Kruskall Wallis. Cani et al (2007) reported that administration of high fat diet along with FOS in mice for 14 days decreased IL-6. Watzl et al. (2005) also reported a decrease of inflammatory process and IL-6 by supplementation of FOS to humans
for three weeks. Obesity is associated with inflammation. Inflamation in turn is associated with raised plasma levels of CRP, TNFα and IL-6 (Lee, 2013). It has been proposed that adipose tissue directly modulate CRP levels.
In humans the major acute-phase reactant protein CRP is synthesized mainly from liver cells by interleukin-6 (IL-6). CRP is then released into the blood (Arnaud et al., 2005).

CONCLUSION

Supplementation of FOS resulted in decrease of CRP (p = 0.000) more than the other two prebiotics but the reduction in IL-6 and visceral fat was more by MOS.

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