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The interplay between oat beta glucan, gut microbiota and gut-liver axis in treatment of obesity associated non-alcoholic steatohepatitis and Type II diabetes mellitus

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Dietary fibers regulate host health through various mechanisms related to their physicochemical structure and physiological properties in the gut. The interplay between diet, gut microbiota and human host appear to play a significant role in pathogenesis of obesity associated complications. This study was designed to unravel oat beta glucan modulatory effect on non-alcoholic steatohepatitis and type II diabetes mellitus in high fat fed rats and to explain possible pathomechanics involving gut microbiota and gut liver axis. Sixty male albino rats were included and randomly divided into four equal groups: control group; positive control group; diet induced obesity group; oat beta glucan treated group. All were subjected to assessment of glycemic profile; liver enzymes; serum trimethylamine-*N*-oxide levels; hepatic G-protein coupled receptor 43 relative gene expression. Histopathological examination of hepatic tissue was performed. Results revealed that oat beta glucan administration improved the biochemical changes. The histopathological findings confirmed the biochemical changes. Gut microbiota appeared to be highly implicated *via* its metabolites short chain fatty acids and trimethylamine. Our conclusion was that oat beta glucan was a successful compliance in the management strategy of hepatic steatosis and diabetes mellitus *via* modulating a number of gut microbial products.

Keywords: Dietary fibers, G-protein coupled receptors, Insulin, Trimethylamine-*N*-oxide

Obesity, is a condition where the stored energy reserve in adipose tissue is increased to the point that impairs health. Obesity has been increasing steadily and is presently at unaccustomed levels affecting all ages and both sex groups irrespective of the geographical locality, ethnicity or socioeconomic level[1]. Obesity raises the risk of a wide array of metabolic diseases as non-alcoholic fatty liver disorders (NAFLD), type II diabetes mellitus (DM) and atherosclerosis. Therefore, the incidence and etiology of NAFLD and Type II DM are intimately linked where obesity associated lipotoxicity and its related consequences are highly involved in pathogenesis of both conditions[2,3,4].

Gut microbiota has attracted increasing attention over the last several decades. In fact, gut microbial profile seems to play a role concerning obesity being changed between lean and obese individuals[5]. Studies carried out on the gut microbiota and gut-liver axis stated that dysbiosis of gut microbiota could be implicated in DM and NAFLD pathogenesis and progression. The most accepted mechanism is bacterial product-induced responses including pro/anti-inflammatory and toxic products as Trimethylamine-*N*-oxide and short chain fatty acids[6,7].

Owing to the growing threat of obesity and the global trend of prebiotic fibers use, this work was designed to detect oat beta glucan modulatory effect on non- alcoholic steatohepatitis and DM in high fat fed rats. High fat diet (HFD) is a gold-standard for studying the interaction between diet, microbiota and disease. HFD induces elevation of microbial dietary energy harvesting that in turn affect body mass index, serum triglyceride level inducing adiposity and fatty liver infiltration. Additionally, HFD triggers a bloom of endotoxin-rich bacteria, mucosal inflammation and barrier dysfunction, resulting in gut-to-systemic endotoxin translocation precipitating steatosis, IR and DM[8,9].

TMAO is a small amine oxide generated enzymatically from dietary choline, betaine and carnitine by coupling of the reactions catalyzed by gut microbiota TMA-lyase and the hepatic enzyme flavin containing monooxygenase-3 (FMO3), respectively. Divulging evidences revealed TMAO role in pathogenesis of atherosclerotic cardiovascular disease. However, the role of TMAO in NAFLD pathogenesis and glycemic

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control still incompletely obvious[7]. In this situation, TMAO was assessed to solve the controversy around its role in NAFLD and type II DM. Is it harmful, protective or diagnostic biomarker? At the same time it is an exact example for integrated gut and hepatic metabolism trying to mediate the harmony between the two biosystems.

Concerning short-chain fatty acids (SCFAs), they are soluble dietary fibers fermentation products that regulate various metabolic processes. SCFAs are thought to exert a beneficial impact on obesity associated insulin resistant state and non-alcoholic steatohepatitis (NASH)[10]. G- protein coupled receptors GPR41 and GPR43 are proposed to have the potential to modulate inflammation and to mediate the interaction between the human host and gut microbiota by binding SCFAs. However, whether these receptors are protective or causative is inconsistent between studies[11,12]. In this situation, we investigated the effect of oat beta glucan enriched formula on hepatic GPR43 expression trying to clarify the effect of gut microbiota generated SCFAs on HFD induced NASH course and DM presenting it as an important link between diet, gut microbiota and disease.

Materials and Methods

The present study was carried out according to the guidance of ethical committee of Medical Research, Faculty of Medicine, Tanta University, Egypt (Approval code: 32286/04/18).

Chemicals

All used chemicals and solvents unless otherwise mentioned were purchased from Sigma Aldrich (Sigma, St Louis, USA).

Experimental animals

This study comprised sixty male albino rats of approximately 120 - 150 g body weight. During the study, animals were housed in wire mesh cages. They were allowed free access to water. Rats were kept under constant environmental conditions (25°C and lighting regimen of 12 h dark/12 h light cycle). Animals were regularly weighed every two weeks.

Experimental design

Rats were randomly divided into four equal groups of 15 rats each (according to the diet they received for a period of 24 weeks) as follows: Group I (Control group) that received a standard diet. Group II (Positive control group) received oat beta glucan enriched formula. Oat beta glucan powder used was purchased from Huzhou Purestar Biochem Company, China. Group III (Diet induced obesity group) received high fat diet (HFD). Group IV (Oat beta glucan treated group) received HFD augmented with oat beta glucan in the same group II dose. Composition of standard and HFD (g/Kg formula) is shown in (Table 1)[13]. A weight of 61.4 g of oat bet glucan powder was added per 1 kg standard and HF dietary formulae for preparation of diets for groups II and IV, respectively[14]. Components of each diet were in turn mixed and pelleted every 2 weeks before their storage at −20°C.

Blood sampling

By the end of the study, all groups' overnight fasted rats were anesthized by ether. With the heart still beating, blood was collected *via* cardiac puncture. An aliquot of 1 mL was preserved on EDTA tube for estimation of glycated haemoglobin (HbA_{1c}) levels while the remaining blood volume was allowed to clot at room temperature then centrifuged at a speed of 3000 rpm for 10 min. Sera were aspirated, aliquoted and finally transferred into dry sterile eppendorf tubes before their storage at −80°C till use.

Tissue sampling

Abdomen was opened; liver was excised and then washed with ice cold saline solution. Tissues were chilled on ice and then divided to small pieces. Portions of hepatic tissue were kept in 10% formosaline to be microscopically examined. The remaining hepatic tissue was wrapped in aluminum foil for storage at −80°C to be used in the molecular study.

Table 1 — Standard and high fat diet composition in grams/each kg.

Components	Standard diet (g)	High caloric diet rich in fat (g)
Wheat flour	772	77
Bran	30	45
Casein	121	321
Palm oil	11	415
Na-acetate	10. 2	15. 3
K_2HPO4	6	9
Na_2H_2PO4	4. 8	7. 2
NaCl	0. 64	0. 96
Vitamin supplements	5	7
Water	39. 4	102. 5
Total weight	1000	1000
Protein (*w/w*)	17	34
Lipid (*w/w*)	2	42. 5
% of calories of protein	22	22
% of calories of lipid	9	72

Biochemical assay

Fasting serum glucose level

Fasting serum glucose level was assessed by colorimetric assay (Spinreact Company, Girona, Spain).

Glycated haemoglobin (HbA1c) levels

HbA_{1c} levels were determined by an enzyme-linked immune-sorbent assay (ELISA) using a commercially available kit supplied by Sun Red Biotechnology Company, Shanghai, China (Catalogue number #: 201-11-0585).

The levels of fasting serum insulin

Fasting serum insulin levels were determined by an enzyme-linked immune-sorbent assay (ELISA) using a commercially available kit supplied by Sun Red Biotechnology Company, Shanghai, China (Catalogue number #:201-11-070).

HOMA-IR calculation

HOMA-IR values were calculated using fasting serum insulin level (ng/mL) and fasting serum glucose level (mg/dL) divided by 405 according to Duseja *et al*[15].

Serum aspartate transaminase (AST) and serum alanine transaminase (ALT) activities

Activities were determined by colorimetric assay according to Tietz, (1976)[16].

Serum Trimethylamine N-oxide (TMAO) level was assessed by colorimetric assay.

Analysis involved chemical reduction of TMAO into Trimethylamine (TMA) using both ferrous sulphate and disodium ethylenediaminetetraacetic acid[17].

Hepatic tissue G-protein coupled receptor 43/free fatty acid receptor 2 (GPR43/FFAR2) gene relative expression was assessed using quantitative real time PCR technique (qRT-pcr)

Total RNA was extracted from frozen hepatic tissue samples after processing using PureLink® RNA Mini Kit (Life Technologies Corporation, USA). The purity, quality and concentration of extracted mRNA were determined using the NanoDrop Spectrophotometer (analyticajena model scandrop, Germany). Synthesis of the first strand was performed with Fast Gene 55-Scriptase Complementary DNA (cDNA) synthesis kit (Nippon genetics Europe, LS-61). cDNA amplification was carried out using SensiFAST™ SYBR® Lo-ROX Kit (Bioline Reagents Ltd, United Kingdom, BIO-94005). GPR43 mRNA transcripts were quantified, relative to the constitutive gene, Glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH). Sequence specific primers were designed as follow: Rat GPR43 (Gene Bank Accession No. NM_001005877.1): forward primer (5'-CTACGAGAACTTCACCCAAGAG -3') and reverse primer (5'-GAAGCGCCAATA ACAGAAGA TG -3') and rat GAPDH (Gene Bank Accession No. NM_0017008.4): forward primer (5'-CTCCCACT CTTCCACCTTCG -3'), reverse primer (5'-GCCT CTCTTGCTCAGTGTCC -3'). Cycling conditions were as follow: Initial activation step at 95°C for 2 min, followed by 40 denaturation cycles at 95°C for 5 s, annealing at 60°C for 10 s and finally extension at 72°C for 20 s. Results were calculated from cycle threshold (Ct) of the target genes which were normalized with (Ct) of housekeeping gene by Step Oneplus real-time thermal cycler (Applied Biosystems, Life technology, USA) and its software then fold change was calculated using the 2-ΔΔCt method[18].

Histopathological study

Hepatic tissue samples preserved in 10% neutral buffered formalin were fixed in paraffin. Sagittal sections of 5 μM thickness were stained with hematoxylin and eosin (H&E), examined at different magnification powers by Olympus BX51 light microscope (Olympus Optical Co. ltd. Tokyo, Japan) and its related camera (Olympus DP50; Olympus Optical Co. ltd. Tokyo, Japan).

Statistical analysis

Statistical analysis was performed as mean±standard deviation (SD) using SPSS version 23. One-way analysis of variance (ANOVA) was used for the multiple comparisons, determination of oat beta glucan efficacy and to evaluate the statistical significance between the different studied groups followed by Tukey's post-hoc test.

Results

Effect of oat beta glucan supplemented diet on body weight (g)

The final body weight was significantly decreased in oat beta glucan treated group as compared to the other studied groups including the high fat induced obesity group. However, there was insignificant difference as regard the final body weight between negative control and positive control groups. Interestingly, using the two-way ANOVA oat beta glucan treatment, as an independent variable, ended in a significant decrease in body weight (Table 2 & Fig. 1)

Effect of oat beta glucan on glycemic profile (Fasting serum glucose; HbA_{1c}; Insulin; HOMA-IR)

HFD administration resulted in a significant rise in fasting serum glucose, serum insulin, HbA_{1c} levels and HOMA-IR values while diet supplemented with

oat beta glucan exerted the reverse effect. Interestingly, by using the two-way ANOVA oat beta glucan treatment, as an independent variable, caused a significant decrease in the assessed glycemic profile parameters (Table 2 and Figs 2A-D).

Effect of oat beta glucan of liver enzymes (AST; ALT)

HFD administration induced a statistically significant increase in serum AST and ALT enzymatic activities while oat beta glucan consumption resulted in significant decrease. Moreover, oat beta glucan treated group showed statistically significant increase in serum AST, ALT enzymatic activities when compared to each of the control groups. Using the two-way ANOVA test enabled identification of oat beta glucan as an inducer of significant decrease in both ALT and AST enzymatic activities (Table 2 and Figs 2E & F).

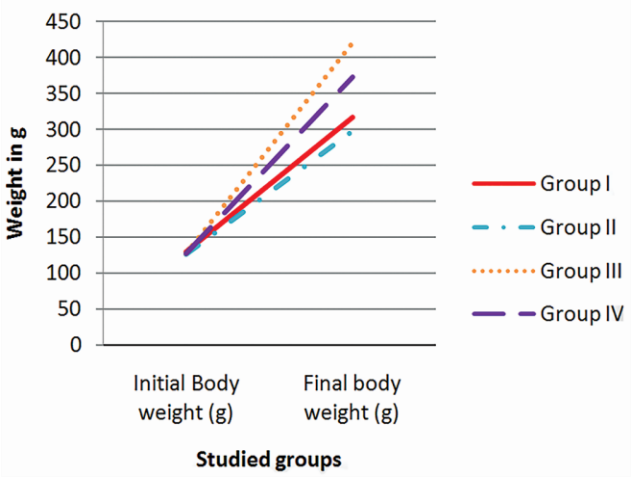

Fig. 1 — Comparison among the studied groups as regard body weight changes (g)

Effect of oat beta glucan on serum TMAO level

HFD administration either with or without beta glucan administration resulted in a statistically significant increase in serum TMAO level. Using the two-way ANOVA oat beta glucan treatment, as an independent variable, caused a significant decrease in serum TMAO level (Table 2 and Fig. 3A).

Effect of oat beta glucan on hepatic GPR43/FFAR2 relative gene expression

HFD administration resulted in significant decrease in hepatic GPR43/FFAR2 relative gene expression as compared to each of the control groups. However, addition of oat beta glucan to diet (either normal or HFD) caused significant increase. Interestingly, by using the two-way ANOVA oat beta glucan treatment, as an independent variable, caused a significant increase in hepatic GPR43/FFAR2 relative gene expression level (Table 2 and Fig. 3B).

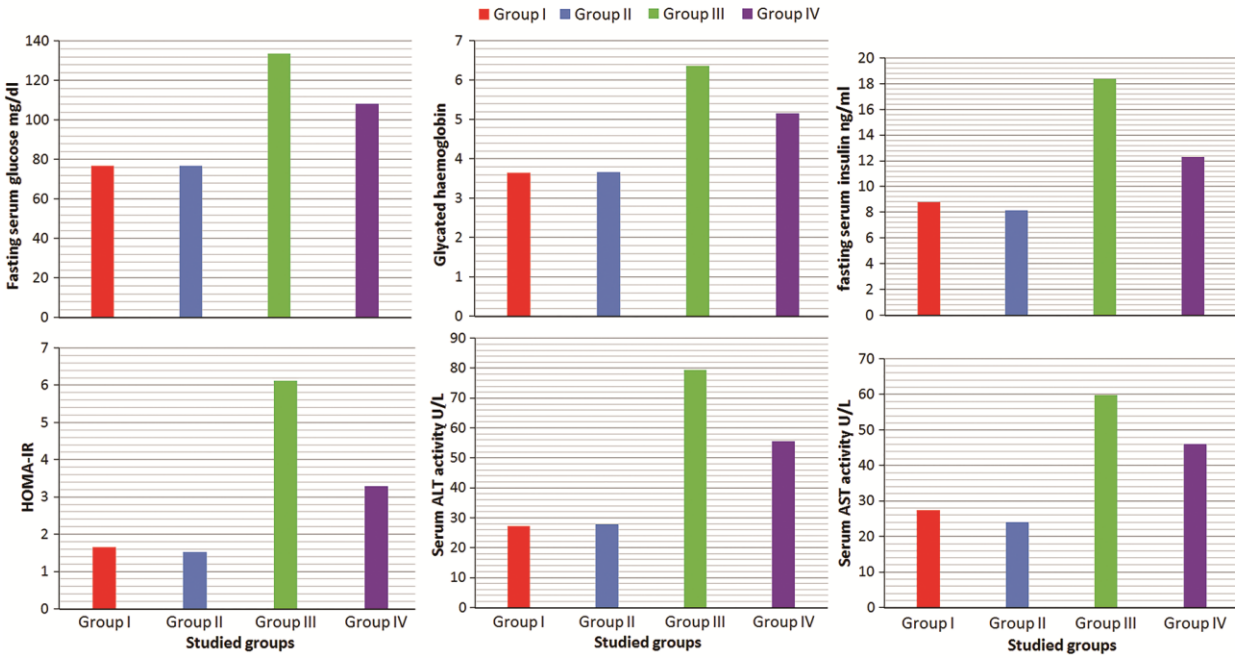

Fig. 2 — Comparison among the studied groups as regard (A) fasting serum glucose level (mg/dL); (B) HbA_{1c} concentration; (C) insulin level (ng/mL); (D) HOMA-IR value; (E) serum aspartate transaminase (ALT) activity (U/L); and (F) serum aspartate transaminase (AST) activity (U/L)

Histopathological study results

Histopathological examination of control group rats' hepatic tissue revealed normal hepatic architecture (Fig. 4). Liver tissue of obese rats microscopically showed macro vesicular steatosis, ballooned hepatocytes with numerous cytosolic fat vacuoles and peripheral nucleus, portal inflammatory infiltrate and lobular inflammation (Figs 5 & 6). Moreover, oat beta glucan treated group showed minimal steatosis with minimal degree of inflammatory infiltration (Fig. 7).

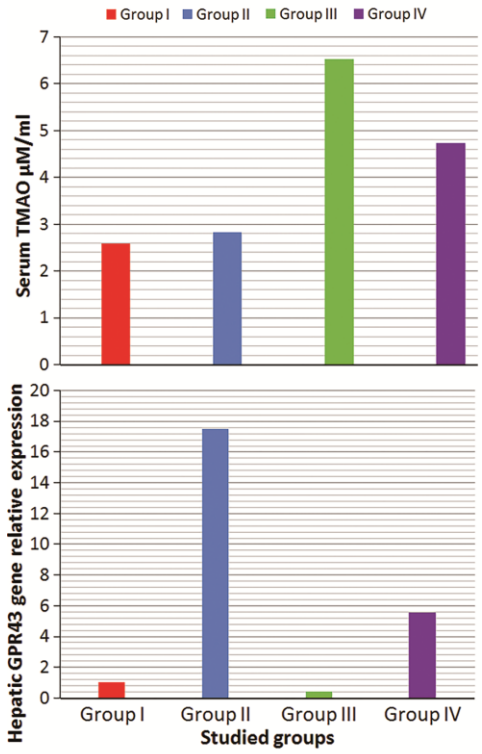

Fig. 3 — Comparison among studied groups as regard (A) Serum TMAO level (µmol/mL); and (B) Hepatic GPR43 gene relative expression

Results & Discussion

Food is not only a primordial need for survival but it could modulate the gut microbial ecology. Key gut microbial metabolites including SCFAs and TMAO are proposed to regulate a variety of physiologic

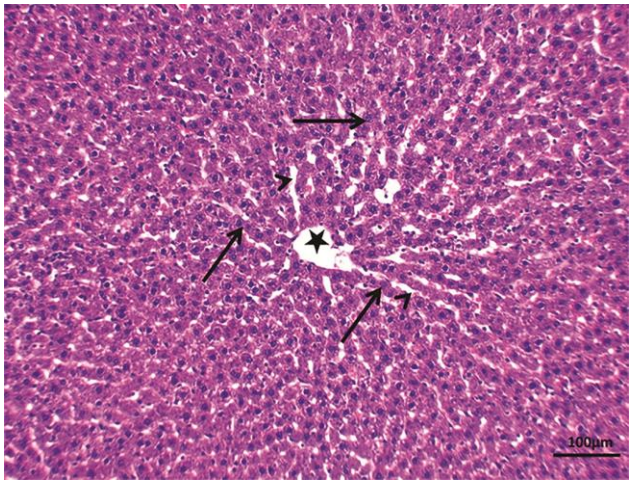

Fig. 4 — A photomicrograph showing the normal group I rats hepatic architecture with a central vein (star) surrounded by radiating cords of hepatocytes (arrows) and separated by the blood sinusoids (arrow heads). Notice, the hepatocytes have granular cytoplasm and large rounded central vesicular nuclei (H&E 200X).

Table 2 — Effect of oat beta glucan on body weight, the assessed biochemical and molecular parameters between all the studied groups

Parameter/group	Group I (n=15)	Group II (n=15)	Group III (n=15)	Group IV (n=15)
Final body weight (g)	316. 839±33. 244^{c,d*}	297. 499±18. 624^{c,d*}	420. 063±29. 875^{a,b,d*}	372. 830±17. 491^{a,b,c*}
Fasting serum glucose level (mg/dL)	76. 393 ± 10. 694^{c,d*}	76. 445± 13. 386^{c,d*}	133. 180±11. 669^{a,b,d*}	107. 955± 8. 972^{a,b,c*}
HbA1c concentration (%)	3. 637± 0. 509^{c,d*}	3. 640 ± 0. 637^{c,d*}	6. 341± 0. 555^{a,b,d*}	5. 141± 0. 428^{a,b,c*}
Serum insulin level (ng/mL)	8. 775 ± 1. 217^{c,d*}	8. 093 ± 0. 797^{c,d*}	18. 311± 3. 675^{a,b,d*}	12. 273 ±2. 874^{a,b,c*}
HOMA-IR	1. 637 ± 0. 211^{c,d*}	1. 512 ± 0. 202^{c,d*}	6. 104± 1. 652^{a,b,d*}	3. 279± 0. 834^{a,b,c*}
Serum ALT activity (U/L)	27. 105 ± 5. 085^{c,d*}	27. 559 ± 7. 331^{c,d*}	79. 049 ± 7. 591^{a,b,d*}	55. 321 ± 6. 660^{a,b,c*}
Serum AST activity (U/L)	27. 133 ± 7. 308^{c,d*}	23. 767 ± 6. 478^{c,d*}	59. 557 ±20. 040^{a,b,d*}	45. 883 ± 6. 943^{a,b,c*}
Serum TMAO level (µmol/ mL)	2. 578 ± 0. 819^{c,d*}	2. 817 ± 0. 890^{c,d*}	6. 514 ± 1. 750^{a,b,d*}	4. 719 ± 2. 741^{a,b,c*}
Hepatic tissue GPR43/FFAR2 relative gene expression	1. 000± 0. 009^{b,d*}	17. 442 ± 2. 713^{a,c,d*}	0. 408 ± 0. 165^{b,d*}	5. 540 ± 4. 510^{a,b,d*}

a-d: significant difference between the study groups at $P< 0.05$*. n: number of experimental rats. Data are mean + standard deviation (SD). Comparative statistics were carried out using the one way ANOVA test and Tukey's post hoc test. [a]denotes the significance from control group (Group I). [b]denotes the significance from positive control group (Group II). [c]denotes the significance from HFD induced obesity group (Group III). [d]denotes the significance from beta glucan treated group (Group IV). HbA1c: glycatedhaemoglobin; ALT: alanine transaminase; AST: aspartate transaminase; TMAO: Trimethylamine-N-oxide; GPR43/FFAR2: G protein coupled receptor 43

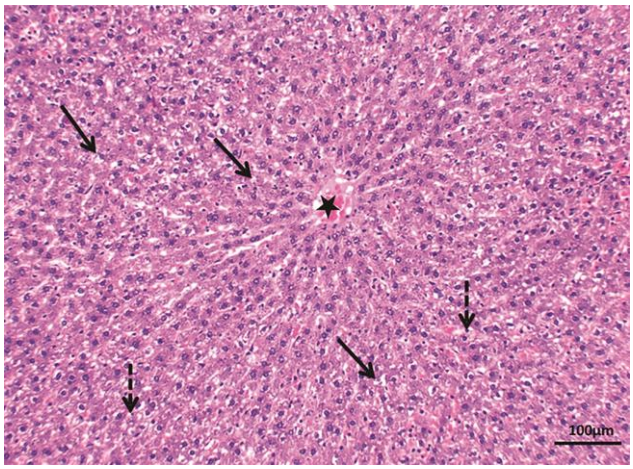

Fig. 5 — Nonalcoholic steatohepatitis (NASH) in group III liver showing macrovesicular steatosis; vacuolated hepatocytes with shrunken peripheral nuclei (arrows). Ballooned hepatocytes (dashed arrows) and congested central vein (star) could be seen (H&E 200X)

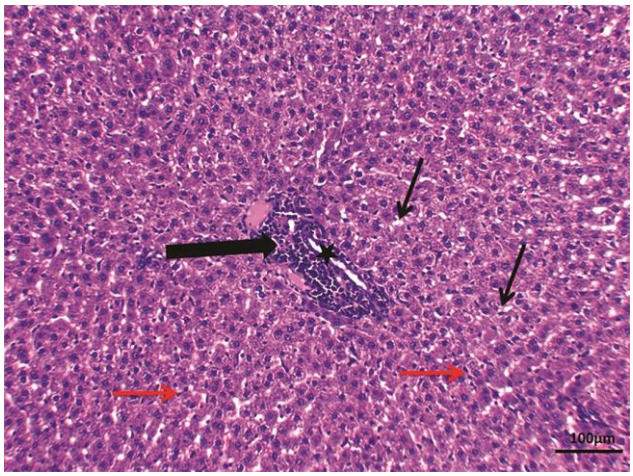

Fig. 6 — A photomicrograph shows a prominent inflammatory infiltrate (thick arrow) surrounding the central vein (star) as well as numerous mononuclear infiltrates (red arrows) in-between hepatocytes. There is evidence of macrovesicular steatosis (thin arrows) (H&E 200X)

processes and to modulate inflammation[19], hence our work was designed to elucidate the role played by oat beta glucan in modulating the gut microbial communities *via* its effect on SCFAs and TMAO generation and to clarify the interplay between these biomolecules in the course of HFD induced type II DM and NASH either by attenuation or augmentation.

The administrated dietary formula succeeded in obesity induction in HFD fed albino rats. Basically after 24 weeks, the body weight of the rats was greatly increased when compared to the standard diet fed and positive control group rats. Our results were in line with Tung *et al*[20]. On the other hand oat beta glucan co-

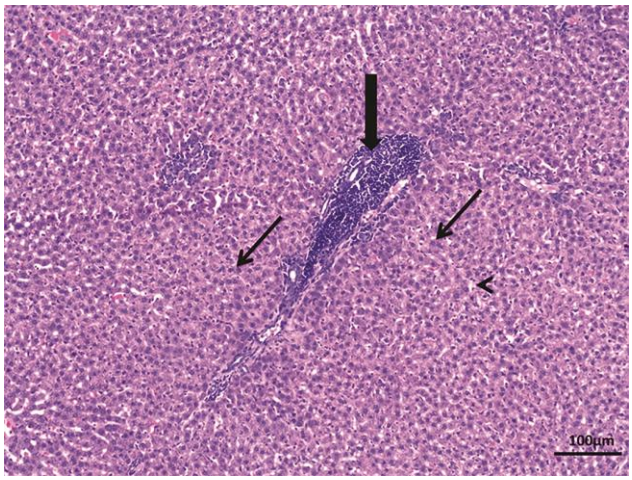

Fig. 7 — (Group IV) showing restoration of normal hepatic architecture with apparent normal hepatocytes (thin arrows). But, inflammatory infiltration (thick arrows) around the central vein and few macrovesicular steatosis (arrow head) could be seen (H&E 200X)

administration with HFD induced a significant reduction of rats' body weight supporting the notion that that there is an inverse relationship between soluble fibers intake and weight gain. This finding was in agreement with Zheng *et al*.[21] and Mosa *et al*[22].

Regarding the glycemic profile, high fasting serum glucose, HbA_{1c}, insulin and HOMA-IR values in HFD fed rats indicated development of IR and type II DM. The exact mechanism could be activation of JNK pathway and serine phosphorylation of insulin receptor substrate-1(IRS-1), inhibition of glucose transporter 4 (GLUT4) translocation and defective glucose uptake[3]. Moreover, increased fatty acids oxidation decreases glucose utilization resulting in more hyperglycemia, hyperinsulinaemia and insulin receptors down-regulation generating a state of IR and in turn DM[3]. Our findings were consistent with Small *et al*.[23] and Kotharie *et al*.[24]. Concerning the effect of oat beta glucan on glycemic profile, our findings were in accordance with Zheng *et al*.[21] and Zhu *et al*.[25] This effect could be explained on basis of oat beta glucan ability to increase satiety and to activate phosphatidyl inositol triphosphate/Akt (PI3K/Akt) pathway relocating GLUT4 to cell surface and stimulating hepatic glycogenesis. Moreover, oat beta glucan has been validated to increase incretins secretion that in turn increase insulin release from pancreatic beta cells adjusting hyperglycemia creating a link between diet, gut, liver and pancreatic beta cells[26].

Our work depicted that HFD induced significant elevation of both ALT and AST enzymatic activities

while oat beta glucan induced significant decrease in their activities. This was associated with a notable reduced count of hepatic fatdroplets, degree of necrosis and inflammatory foci in comparison to obese rats that experienced severe steatosis on hepatic histopathological examination. Our results were in agreement with Hosseini *et al*. who documented that ALT and AST enzymes had significant decrease in the experimental group received barely compared to the group receiving cholesterol supporting the role of cereal beta glucan whatever the source in reducing the risk of NASH constituting another connection between diet, gut and liver function[27].

TMAO has been suggested to be another link between diet, gut microbiota, hepatic FOM3 and illness. Several research works point to the relationship between elevated serum TMAO level and increased risk of cardiovascular diseases, DM and even cancer[28]. The present work unraveled that serum TMAO level is significantly elevated in HFD induced obesity group when compared to the other studied groups including the treated group. This was intimately linked to IR, hepatic steatosis and down regulation of GPR43/FFAR2 relative expression in hepatic tissue. This was in accordance to Oellgaard *et al*.[29] and Zhuang *et al*.[30]. Chen *et al* documented that TMAO effect "on the glycemic profile could be *via* activation of PERK, a novel endoplasmic reticulum stress kinase, in pancreatic beta cell[31]. Furthermore Tan *et al.*, stated that TMAO aggravated hepatic steatosis *via* suppression of bile acid mediated hepatic farnesoid × receptor signaling[32].

As regard GPR43 relative gene expression, our results revealed that beta glucan significantly increased GPR43 expression as previously reported by Lu *et al*[33]. In addition Maruta and Yamashita reported that differentiated myotube cells GPR43 expression was increased upon acetic acid administration[34].

In our work GPR43/FFAR2 overexpression was associated with reduction of body weight, alleviation of IR, improvement of liver enzymes activities, reduction of serum TMAO levels and amelioration of hepatic histopathological alterations in treated group rats. Kobayashi *et al*. revealed that SCFAs anti-inflammatory effect was exerted *via* inhibition of TNF-α-induced MCP-1 expression by modulating p38 and JNK signaling pathways. This was validated by blocking of SCFA-mediated inhibition of MCP-1 expression by siRNA-induced gene silencing of GPR41and GPR43[35]. Similar results were reported by Kimura *et al*[36]. These finding came in line with our observation and support the notion that SCFAs exert their anti-inflammatory effect partly *via* GPR43 signaling.

Not only the anti-inflammatory effects but also their effect on body weight and tissue metabolism could be exerted *via* such receptor. This was obviously supported by Kimura *et al*. whose explanation was that GPR43 activation suppressed insulin signaling in adipocytes, which in turn inhibited fat accumulation in adipose tissue and promoted the metabolism of unincorporated lipids and glucose[36]. In addition, GPR43 overexpression in the intestine improves glucose tolerance by promoting the secretion of incretins from L cells as reported by Gad *et al*[26]. These effects on body weight and carbohydrate metabolism were also evident in our work.

The role of SCFA on TMAO generation has been not fully elucidated in literature. SCFAs could change the microbial milieu reducing TMA biosynthesis and thus TMA amount delivered to liver reducing TMAO biosynthesis and circulating levels. SCFAs may reduce expression or block the activity of either intestinal TMA lyase and/or hepatic FMO3. From our point of view, this is an important interconnection point between diet, intestinal microbiota and hepatic enzymatic activity. However further studies are required to support this notion.

Based on our results, we consider GPR43 as a drug target and a possible way for connecting diet, gut, liver, and illness relief. We propose that future studies on human tissues, *ex vivo*, will support the role played by GPR43, SCFAs and TMAO in human disease, be a causative or protective.

Conclusion

Assessed data stated that HFD downregulated hepatic GPR43/FFAR2 gene expression while oat beta glucan upregulated it. This was reflected on the estimated biomarkers and the histopathological findings confirming the effective outcome of potential use of oat beta glucan as in management of obesity associated complications including type II DM and NASH and the ability of such fibers to link diet, gut and liver *via* various mechanics.

Conflict of interest

All aurthors declare no conflict of interest.

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