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RESEARCH ARTICLE

Prebiotics Supplementation Ameliorates High Fat High Sugar Diet-Associated Oxidative Stress

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ABSTRACT

High fat high sugar (HFHS) diet results in various disorders including oxidative stress. In present study, prebiotics supplementation was given to rats following HFHS diet feeding. The results showed that prebiotics significantly lowered the HFHS-diet associated elevated levels of cholesterol, triglyceride, low density lipids, alkaline phosphatase, blood urea, creatinine, uric acid and total proteins. Prebiotics significantly restored the HFHS-diet induced decrease in total anti-oxidant capacity. The levels of alanine aminotransferase, aspartate aminotransferase, bilirubin, total oxidation status, malondialdehyde, paraoxonase and arylesterase were not significantly different in HFHS-Prebiotics group as compared to control group. Histological analyses of liver, intestine and kidney tissues in HFHS-group showed cytoplasmic vacuolation, mucosal damage, hepatic triad abnormalities, eccentric nuclei, focal necrosis, tubular congestion and neutrophil infiltration which were significantly improved in HFHS+Prebiotics group suggesting ameliorative potential of prebiotics. In conclusion, our results demonstrated that prebiotics possess therapeutic potential in ameliorating HFHS-diet associated alterations in metabolic profile, oxidative stress markers and histological architecture in intestine, liver and kidney tissues.

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INTRODUCTION

Excessive intake of dietary fat increases fat accumulation in general circulation, liver and adipose tissue leading to obesity and metabolic diseases (Lambert *et al.*, 2015). HFHS-diet increases ectopic fat accumulation, obesity, oxidative stress, inflammation, kidney and liver damage (Echeverría *et al.*, 2018). Obesity and excessive visceral fat results in dysregulation of tumor necrosis factor α (TNF-α), lipopolysaccharides (LPS), reactive oxygen species, inflammatory mediators (interlukin-6 and interlukin-8) and anti-inflammatory mediators (adiponectin) (Cani and Delzenne, 2009).

Gut microbiota derived-lipoploysaccharide (LPS) is considered as potent inflammatory inducer in mediating the progression of metabolic diseases (Chappuis *et al.*, 2017). The gut microbiota harvests the energy from carbohydrates and contributes in host metabolism. Fermentation of carbohydrates, which are not absorbed from upper gastrointestinal tract, produce essential volatile fatty acids (acetic acid, propionic acid, butyric acid) and organic acids (succinate, pyruvate, lactate) (Hira *et al.*, 2018). Gut-dysbiosis, imbalance in gut microbiota such as Firmicutes/Bacteroidetes ratio, occurs with excessive intake of HFHS-diet (Zhou *et al.*, 2014). The increased dietary fiber contents in food elicit many physiological processes not in gut, but also systematically (Bindels *et al.*, 2015). Dietary fibers influence gut microbiota resulting in gut-associated changes like gut barrier function, endocrine function, metabolism and nitrogen cycle. These changes affect the biochemical and physiological processes of detoxification organs including liver and kidneys (Kieffer *et al.*, 2016).

Prebiotics decrease *de novo* lipogenesis by minimizing the level of acetyl co-A carboxylase (ACC), fatty acid synthase, sterol-responsive element-binding

protein, carbohydrate responsive element binding protein, non-esterified fatty acids and serum lipid by acting on gut mucosa (Delzenne *et al.*, 2013). The treatment of metabolic diseases is being planned with weight loss and energy balance, although no surgical and medicinal therapies are still suggested (Boursier *et al.*, 2016). In this study, prebiotics potential, as dietary intervention and economic approach, is studied for management and treatment of HFHS diet-related hypercholesterolemia and oxidative stress in rats.

MATERIALS AND METHODS

Prebiotics: The product Impim, composed of dandelion fluid extract and glycyrrhiza fluid extract containing glycyrrhizin (0.1%), total flavonoids (2.0%), flavone luteolin and liquiritin, was procured from Keep Young Company, China. Dosage @300mg/kg feed was calculated from the previous literature (Asha *et al.*, 2017).

Experimental design: Twenty four male albino rats at the age of 4-week were purchased and kept according to standard conditions 25°C, 12h alternate light and dark cycle, *ad-libitum* access to diet (Table 1) and water at animal house of Institute of Physiology and Pharmacology, University of Agriculture, Faisalabad.

Table 1: Diet composition

Feed constituents	Normal diet (%)	High Fat and High Sugar (HFHS) diet (%)
Fat	6	36
Sucrose	Nil	40
Crude protein	20	8.75
Crude fiber	4.5	1.23
Ash	6	0.9
NFE	63.5	13.12

The 1st group was kept as control group and provided *ad-libitum* access to normal diet and water. The 2nd group was administered prebiotics with standard feed and water *ad-libitum*. The 3rd group was fed high fat and high sugar (HFHS) diet to induce metabolic hepatitis. The 4th group was given HFHS diet along with prebiotics.

Serum biochemical analysis: The stored serum was thawed and analyzed for cholesterol, triglycerides, low density lipids (LDL), high density lipids (HDL), bilirubin, alanine aminotransferase (ALT), aspartate amino-transferase (AST), alkaline phosphatase (ALP), total protein, creatinine, uric acid and urea levels through commercially available bio-kits (Merck, Pvt, Ltd). Total oxidant status (TOS), total anti-oxidant capacity (TAC), malondialdehyde (MDA), paraoxonase and arylesterase levels were measured by colorimetric method using spectrophotometer (Thermo Scientific Multiskan GO™ with SkanIt software 4.1) according to manufacturer's guidelines.

Tissue analysis: Liver, intestince and kidney samples were washed with normal saline and preserved in 10% neutral buffered formaline before processing for standard histological analysis. Three representative images of intestine, liver and kidneys from each group showing different microscopic fields were taken with the camera (TOUPCAM, ToupTek Photonics Co., Ltd; China) attached to a light microscope (Model IM-910 IRMECO GmbH & Co; Germany). The degree of histopathological alterations were recorded for each group and classified according to the severity such as 0 for normal limits, 1 for minimal, 2 for slight, 3 for moderate and 4 for severe as mentioned in literature (Hussain *et al.*, 2019).

Statistical analysis: The SPSS software (version 16.0) was used for data analysis. One way analysis of variance was applied followed by Duncan's Multiple Range test. All results were expressed as Mean ± SE.

RESULTS

Prebiotics supplementation restored HFHS-diet induced increase in serum lipid levels and ilial histology: As expected, HFHS-diet increased the cholesterol, triglyceride and LDL levels whereas prebiotics supplemenation ameliorated cholesterol, triglyceride and LDL levels. The HDL level decreased significantly in HFHS+Veh group while prebiotics supplementation significantly increased the level of HDL in Prebiotics and HFHS-Pre group (Fig. 1). The histopathological analysis of vehicle group (Left panel) showed normal epithelial lining, villi structure, glands and intestinal mucosa. The HFHS group (Middle panel) showed fat accumulation in ilial region and damaged gut mucosa and villi. The thick epithelium showed pyknotic and eccentric nuclei. The HFHS-Prebiotics group (Right panel) showed rare cytoplasm vacuolation, normal villi and glandular epithelium suggesting ameliorative effects of prebiotics on gut histology (Fig. 1).

Prebiotics supplementation improves HFHS-diet induced alteration in liver function markers: The increased levels of ALT, AST and ALP in HFHS+Veh group indicated hepatic abnormalities. Prebiotics supplementation in HFHS+Pre showed significant decrease in ALP levels, while non-significant difference was noticed on ALT, AST and bilirubin levels. The levels of ALT, AST, ALP, and bilirubin showed non-significant differences in Vehicle *vs* Prebiotics alone group as shown in Fig. 2. The histopathological analysis of vehicle group (Left panel) showed normal hepatocytes, hepatic triad and no fat accumulation. On the other hand, histopathological analysis of HFHS diet-treated group (Middle Panel) showed abnormal hepatic triad, cytoplasmic vacuolation, perivascular and portal cell infiltration, fat accumulation in hepatocytes, eccentric and pyknotic nuclei. The HFHS-Prebiotics group (Right panel) showed restoration of liver parenchyma suggesting ameliorative effects of prebiotics on HFHS diet-induced alterations in liver tissue (Fig. 2).

Prebiotics supplementation alleviates renal damage associated with HFHS-diet: The significantly high level of blood urea, creatinine, uric acid and total protein in HFHS+Veh group indicated abnormal functioning of renal system, while HFHS+Pre group suggested significant ameliorative effects of prebiotics in lowering the levels of blood urea, creatinine, uric acid and total protein. The non-significant difference in levels of blood urea, creatinine, uric acid, and total protein was noticed in vehicle group as compared to prebiotics group (Fig. 3). As

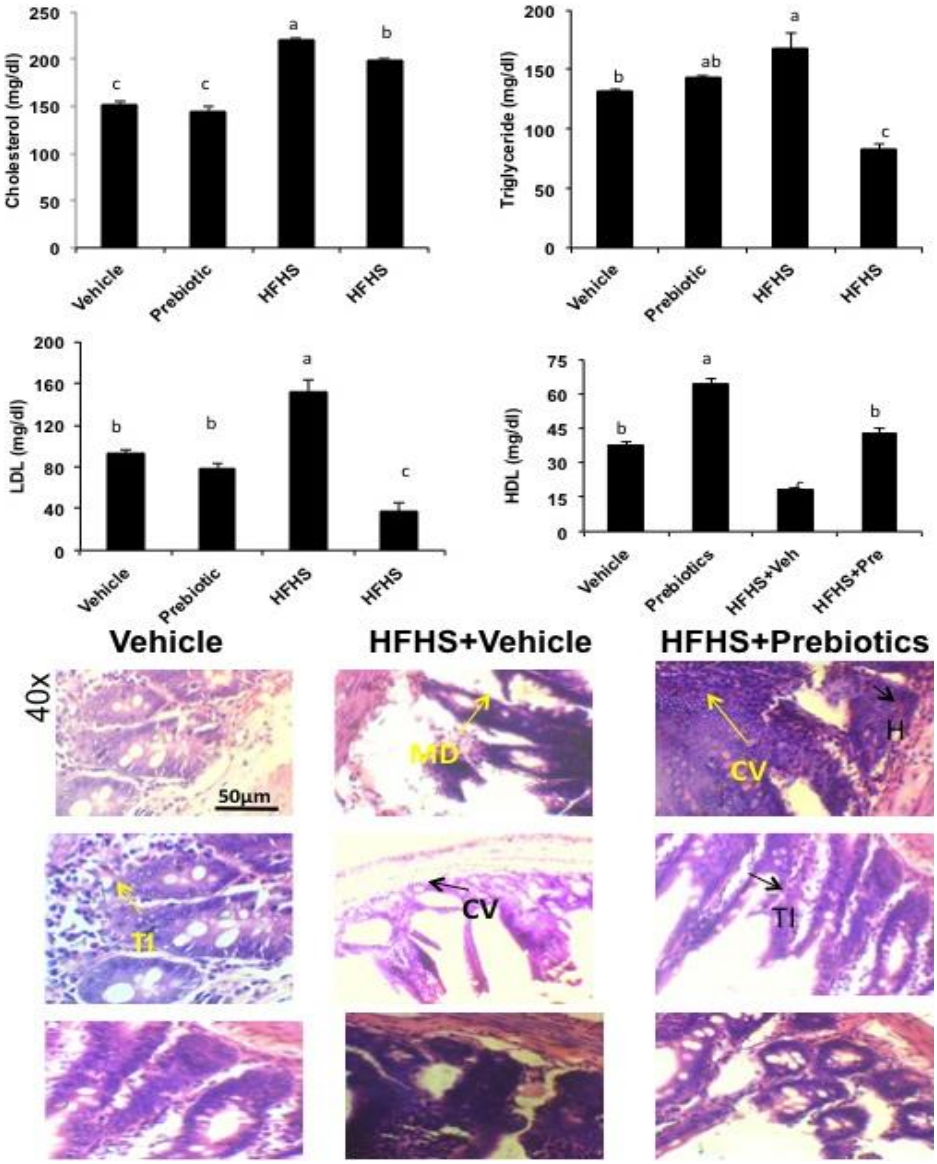
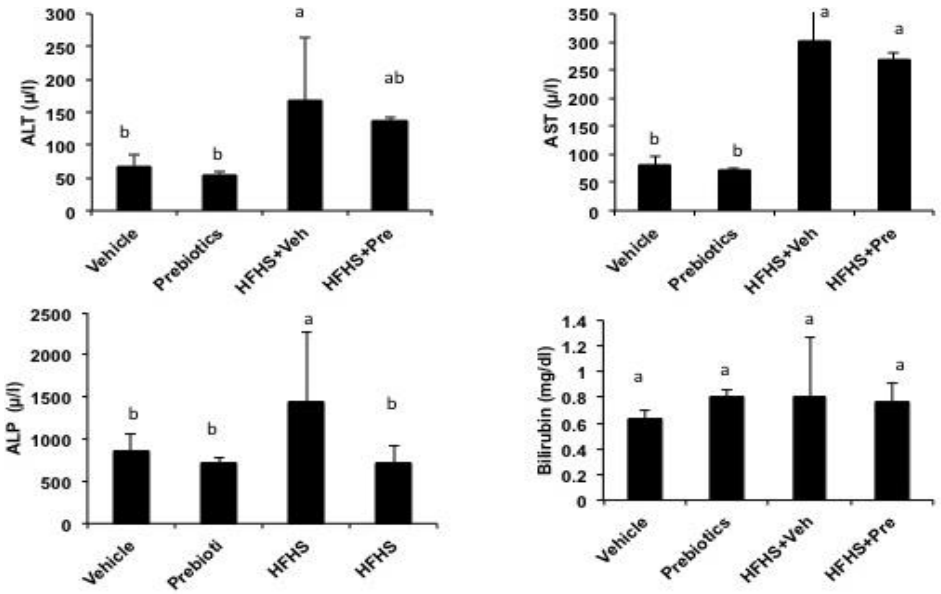


Fig. 1: Effect of HFHS-diet and prebiotics on serum lipid levels and ilium histology. Different alphabets showing statistical significance at P<0.05. Three representative images from respective group showing different areas of ilium. CV, cytoplasmic vacuolation; TI, Thickened intestinal muscle layer; H, Hemorrhages; MD, Mucosal damage.



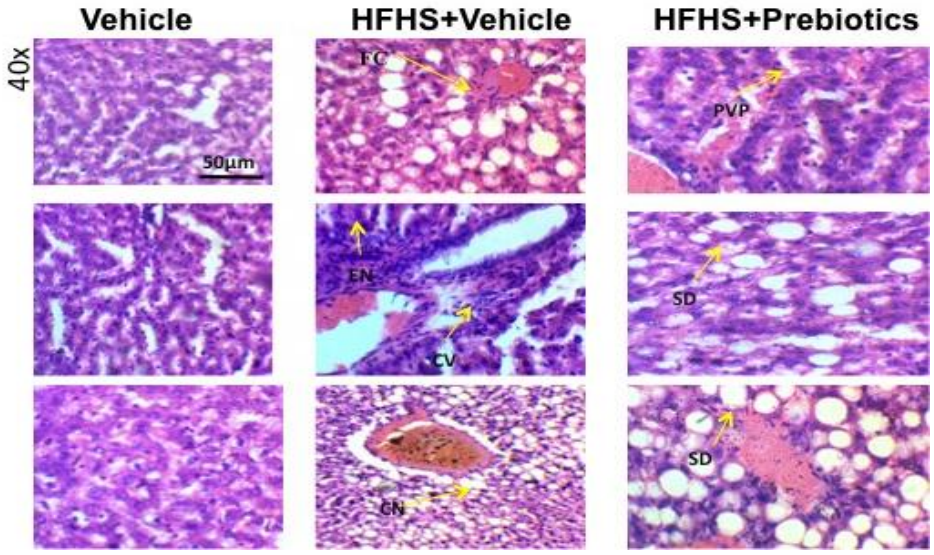


Fig. 2: Effect of HFHS-diet and prebiotics on liver function markers and liver histology. Different alphabets suggest statistical significance at P<0.05. CV, cytoplasmic vacuolation; FC, Focal hepatic necrosis; CN, Centro-lobular necrosis; EN, Eccentric nuclei; SD, Sinusoidal dilation.

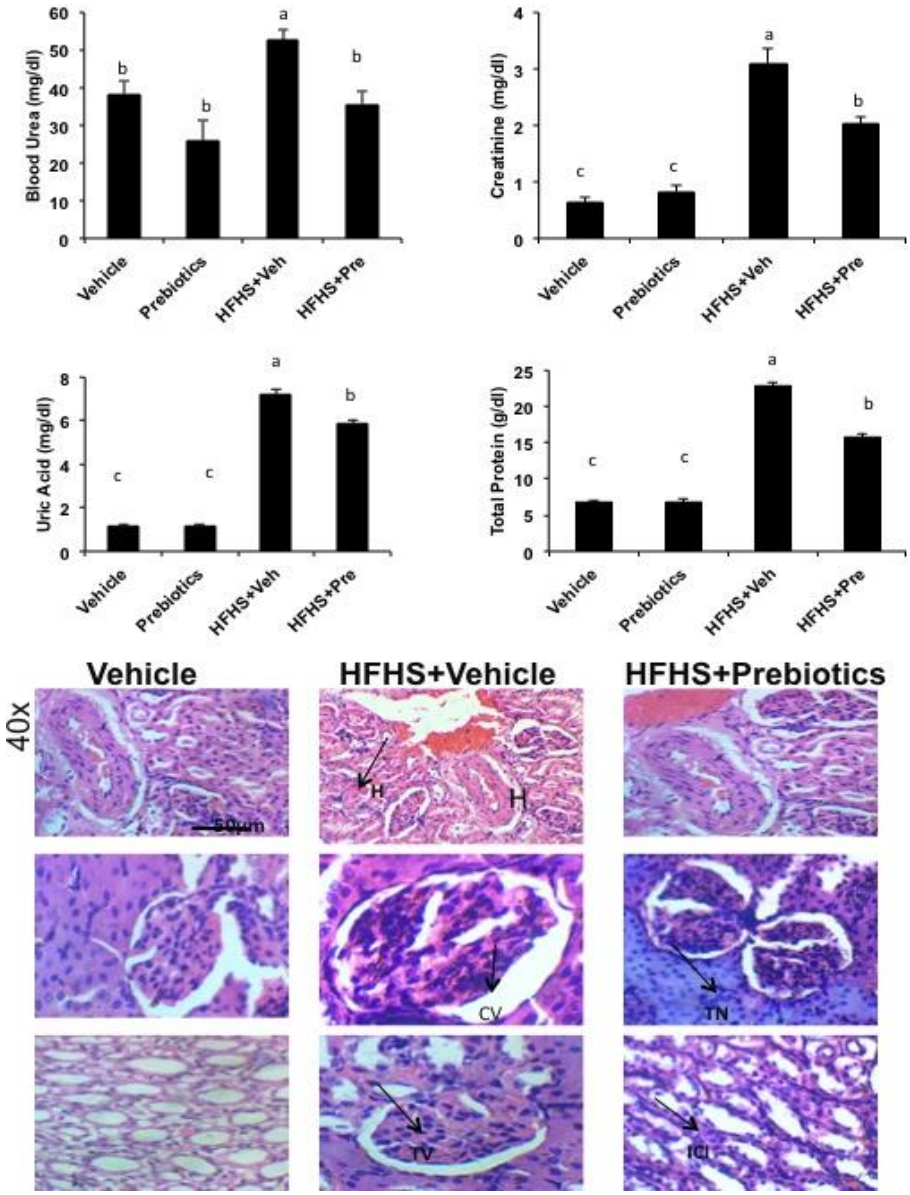


Fig. 3: Effect of HFHS-diet and prebiotics on kidney function markers and kidney histology. Different alphabets suggest statistical significance at P<0.05. CV, cytoplasmic vacuolation; TN, tubular necrosis.

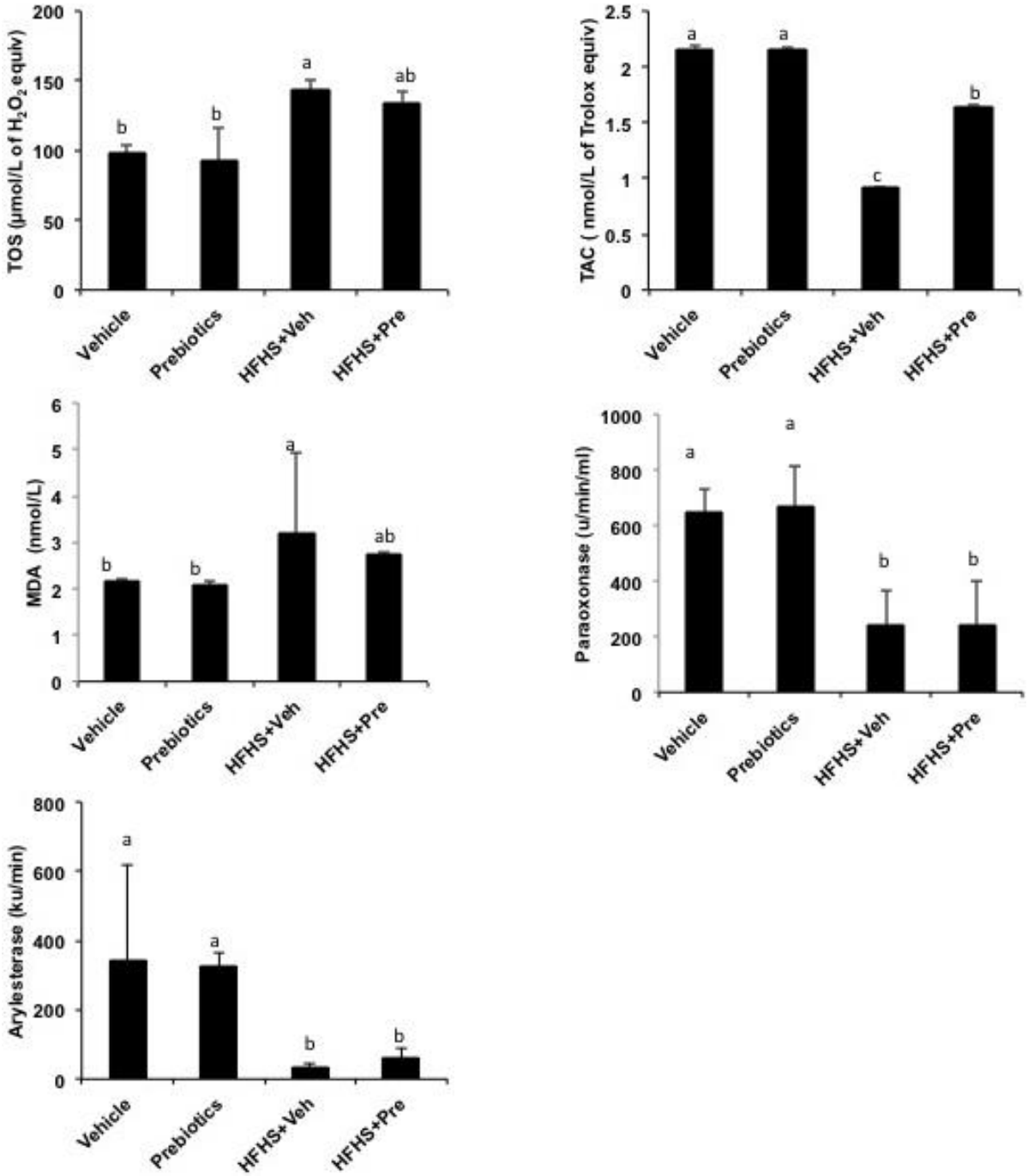


Fig. 4: Prebiotics ameliorate HFHS-diet associated oxidative stress parameters. Different alphabets show statistical significance at P<0.05.

shown in Fig. 3, histopathological analysis of vehicle group (Left panel) showed normal Bowman's capsule and proximal convoluted tubular structure. The HFHS group (Middle panel) showed distorted glomeruli with increased Bowman's capsular space. HFHS-Pre group (Right panel) showed renal architecture comparable to that in vehicle-treated group. In sum, it was found that prebiotics restored not only the serum biomarker levels but also ameliorated the overall renal architecture in HFHS-Pre group as observed by histological analysis (Fig. 3).

Prebiotics supplementation reduces oxidative stress induced by HFHS-diet: HFHS-group showed significantly increased TOS and MDA whereas significantly decreased TAC, paraoxonase and arylesterase levels. Prebiotics supplementation significantly restored HFHS-induced decrease in TAC levels, while the effect was non-significant on TOS, MDA, paraoxonase and arylesterase levels (Fig. 4).

DISCUSSION

Prebiotics are involved in gut-mediated peripheral and luminal metabolism through improving intestinal epithelial junctions and maintaining gut microbiota health (Wilson and Whelan, 2017). Undigested dietary fibers, oligosaccharides and resistant starch are largely fermented in distal colon and produce short chain fatty acids having anti-inflammatory effects in the gut (Froebel *et al.*, 2019). Use of prebiotics in the treatment of obesity and oxidative stress-associated hypercholesterolemia has been previously reported (Tilg and Moschen, 2010). In this

study, we demonstrated the mechanism of beneficial effects of prebiotics in ameliorating gut-liver-kidney axis by studying the biochemical and histological alterations in HFHS-animal model. We show that prebiotics augment overall antioxidant capacity thereby exerting protective effects not only on the liver and kidney function markers but also in restoring the intestinal, liver and kidney histological architecture.

Gut-liver axis plays important role in pathogenesis of NAFLD-associated obesity, oxidative stress and metabolic diseases (Frazier *et al.,* 2011). Chronic consumption of HFHS-diet induces gut dysbiosis (Zhou *et al.,* 2014) facilitating the growth of Gram-negative bacteria particularly *Proteobacteria* species (Jalanka-Tuovinen *et al.,* 2011). The tight junctions of intestinal epithelia loosen up due to lipopolysaccharides originating from Gram-negative bacteria (Jiang *et al.,* 2015). Impaired intestinal barrier 'leaky gut" results in endogenous toxins inflow through hepatic-portal system thereby producing systemic effects (Wiest *et al.,* 2017). Several studies have underscored the importance of prebiotics in improving gut microbiota health and immune functions (Lambert *et al.,* 2015; Chappuis *et al.,* 2017; Wilson and Whelan, 2017). Gut microbiota controls bacterial endotoxins lipopolysaccharides, tumor necrosis factor α, eicosanoids and chemokines (Boulangé *et al.,* 2016).

Lipid homeostasis is regulated through balance between lipolytic and lipogenic pathways (Sanders *et al.,* 2018). Lipid lowering effects of prebiotics supplementation in our study might be due to anti-lipogenic effects of prebiotics through lowering the expression of fatty acid synthase and altering adipocyte morphology (Liu *et al.,* 2017). Moreover, it has been shown that glycemic/insulinemic response is mediated by dietary fibers in prebiotics which stimulate the secretion of glucagon-like peptide-1(GLP-1) implicated in glucose intolerance (Hira *et al.,* 2018).

The elevated levels of ALT, AST and ALP in high fat high sugar fed animals have been reported in previous literature (Echeverría *et al.,* 2018). The possible mechanisms of hepatoprotection by prebiotics supplementation include 1) modulation of fasting-induced adipocyte factor, 2) modulation of farnisoid x receptor for bile acid production, 3) modulation of inflammatory responses through inhibition of bacterial LPS (Vulevic *et al.,* 2013). Moreover, prebiotics increase the production of anti-inflammatory cytokines (IL-10) and decrease inflammatory cytokines (IL-6, IL-1β, TNFα) thereby enhance the innate immune system (Vulevic *et al.,* 2008).

Renal disorder in conjunction with liver disease potentiate the impaired excretion of metabolites and endogenous toxins (Kieffer *et al.,* 2016) resulting in deposition of urates in nephron (Liu *et al.,* 2017). The accumulation of *p*-cresyl sulfate, the prototype of protein-bound uremic toxins produced due to gut dysbiosis, contribute towards insulin resistance, hyperglycemia and glomerulonephropathy (Li *et al.,* 2019; Vitetta *et al.,* 2019).

Oxidative stress is associated with irregular production of adipokines, which in turn mediate metabolic syndrome (Liu *et al.,* 2017; Hussain *et al*., 2019; Ishtiaq *et al*., 2019). The increased levels of oxidative stress markers superoxide dismutase and malondialdehyde indicate peroxidation of unsaturated fatty acids (Patel *et al.,* 2007). Antioxidative effects of prebiotics in current study might be due to reactive oxygen species scavenging properties of prebiotics by inhibition of caveolin signaling, nitric oxide production (Wilson and Whelan, 2017) and modulation of superoxide dismutase and glutathione peroxidase genes expression (D'Souza *et al.,* 2010).

Conclusions: Our results show that HFHS-diet administration in rats during 14 week resulted in significant alterations in biochemical parameters alongwith changes in liver and kidney function markers. Prebiotics supplementation along with HFHS-diet showed ameliorative effects on biochemical profile, liver and kidney function markers.

List of abbreviations: ALT: Alanine aminotransferase. ALP: Alkaline phosphatase. ANOVA: Analysis of variance. AST: Aspartate aminotransferase. CFU: Colony Forming Unit. DMR: Duncan's new multiple range test. H&E: Hematoxylin and eosin. HDL: High-density lipoprotein. HFHS diet: high fat high sugar diet. LDL: low-density lipoprotein. MDA: Malondialdehyde. NAFLD: Non-alcoholic fatty liver disease. SOD: Superoxide dismutase. TAC: Total antioxidant capacity. TOS: Total oxidant status.

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