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Polyphenol rich extracts of finger millet and kodo millet ameliorate high fat diet-induced metabolic alterations

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Finger millet (FM) and kodo millet (KM) are known for their multiple health benefits. Several studies have indicated the antioxidant and hypoglycemic potential of polyphenol rich extracts (PREs) from them. However, the protective roles of PREs from these millets in overcoming high-fat diet (HFD)-induced obesity have not yet been investigated. This study aimed to identify the polyphenols in FM-PREs and KM-PREs using HPLC-DAD/ESI-MS, and to evaluate the role of PREs in mitigating lipopolysaccharide induced inflammation in murine macrophage cells and in the reduction of HFD-induced metabolic complications using male Swiss albino mice. The results suggested that KM-PRE had higher polyphenol content than FM-PRE, of which taxifolin (98%) and catechin (86.6%) were the major fractions respectively. FM-PRE and KM-PRE prevented obesity, however, KM-PRE was more profound in preventing weight gain, adipose tissue hypertrophy, hepatic steatosis, and systemic inflammation than FM-PRE. This study suggests that FM-PRE and KM-PRE could be exploited for developing functional foods or nutraceuticals against obesity and comorbidities.

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1. Introduction

Abnormal weight gain, obesity, and the resultant metabolic complications have emerged as serious health concerns. The prevalence of obesity has tripled from 1975 to 2016 worldwide.[1] According to the WHO, India ranks third after the US and China with the highest number of obese people and ranks second in childhood obesity.[1,2] More than 135 million people are overweight in India.[1,3] Shifts from eating traditional diets that are rich in whole grains to the ones rich in processed grains contributing to excess calories together with the enhanced sedentary lifestyle are considered to be the major contributing factors for this crisis. While anti-obesity therapeutics are helpful, due to their side-effects, diet-based strategies and lifestyle changes have become pivotal preventive measures for tackling these maladies.[4] Among the diet based strategies, diet diversification, inclusion of whole grains in the diet, consumption of dietary fiber and antioxidant-rich diets are being suggested to control weight gain and associated metabolic diseases.[4,5]

Millets, being whole grain cereals, are one of the important domesticated crops since the ancient times of human civilization and are considered among the first cereals in Asia and Africa. There are several types of millets such as proso millet, kodo millet (KM), finger millet (FM), foxtail millet, barnyard millet, little millet, and pearl millet, which have diverse nutritional compositions. Among them, FM and KM have been largely reported to show high antioxidant, hypoglycemic, anti-atherosclerotic, and anti-obesity efficacies.[6–12] KM and FM are good sources of dietary fibers, micronutrients, macronutrients and bioactive phytochemicals especially, polyphenols.[11,12] Polyphenols are a large group of secondary metabolites that possess antioxidant properties and rejuvenated a lot of interest because of their multiple beneficial effects. Polyphenol intake has been shown to alleviate obesity and associated metabolic alterations such as insulin resistance, hepatic steatosis, and cardiovascular complications.[13] The beneficial effects of polyphenols on obesity and metabolic complications are attributed mainly to their anti-inflammatory and antioxidant properties.[13,14] Furthermore, polyphenols have also been

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reported to suppress fat absorption from the gut and enhance the catabolic activities in adipose tissues.[15–18] Several studies have suggested that a regular consumption of FM and KM could prevent diet-induced obesity, insulin resistance and gut dysbiosis.[6,7,9] However, the polyphenols from FM and KM are not yet evaluated for their protective role in high-fat diet (HFD)-induced obesity. Therefore, the present study aimed at the identification of polyphenols in the FM and KM polyphenol rich extracts (PREs), evaluation of the protective power of FM-PREs and KM-PREs against LPS induced inflammation in murine macrophage cell lines (RAW 264.7 cells) and *in vivo* protective efficacy of FM-PREs and KM-PREs against HFD-induced obesity and comorbidities.

2. Materials and methods

2.1 Plant material and reagents

KM JK-65, KM pudu koraipatti, FM Co(Ra)14, and FM allimoyar were grown in the field of National Agri-Food Biotechnology Institute (NABI), Mohali, India. Market varieties of Kodo millet, varagu rice, and Ragi whole finger millet were purchased from big basket (BB Royal organic, Bengaluru). Lipopolysaccharide (LPS) from *E. coli* 055:B5, the Folin–Ciocalteu and phenol standards: gallic acid, vanillic acid, ferulic acid, *p*-coumaric acid, homovanillic acid, naringin, chlorogenic acid, quercetin, – (–) epicatechin, kaempferol, taxifolin, sinapic acid, methyl vanillate, catechin and protocatechuic acid were purchased from Sigma-Aldrich (Sigma-Aldrich Co., St Louis, MO, USA). Fetal bovine serum (FBS) and penicillin/streptomycin (P/S) solution were purchased from Invitrogen (Invitrogen, Camarillo, CA, USA). Dulbecco's modified Eagle's medium (DMEM) was purchased from Zenbio (Zenbio Inc., Research Triangle Park, NC, USA). Methanol, hexane, and other reagents used were of analytical or HPLC grade and were purchased from Central Drug House (CDH, New Delhi, India). Murine macrophage cell line (RAW 264.7) was purchased from National Center for Cell Science (Pune, Maharashtra, India). An ELISA kit for leptin was purchased from Invitrogen (Camarillo, CA, USA); TNF-α and IL-6 kits were purchased from Elabscience (Elabscience Biotechnology Inc., USA); an IL-1β kit was purchased from Ray Biotech (Norcross, GA, USA) and an LPS kit was purchased from Cusabio Biotech (Wuhan, China).

2.2 Preparation of FM-PRE and KM-PRE

FM and KM polyphenol-rich extracts (PREs) were prepared as described earlier by others with slight modifications.[19,20] Briefly, dried millets were cleaned, de-husked, coarsely pulverized and defatted with *n*-hexane for 3 h using a Soxhlet extraction unit (45 °C). Defatted materials were dried and refluxed overnight with methanol in the Soxhlet apparatus. This extraction process was repeated three times and the extracts were pooled. The methanol extracts were concentrated using a rotary evaporator under vacuum at 40 °C. The concentrated extracts were freeze-dried by lyophilization and the lyophilized powders were stored at −80 °C for further use.

2.3 Determination of total phenolic, flavonoid, and tannin contents

The total polyphenol content (TPC) was estimated by the Folin–Ciocalteu method using gallic acid as the standard and the results were expressed in mg GAE (gallic acid equivalents) per g.[21] The total flavonoid content (TFC) was determined by the $AlCl_3$ method using quercetin as the standard and the values were expressed in mg QE (quercetin equivalents) per g.[22] The total tannin content (TTC) was determined by the vanillin–HCl method.[11] Catechin was used as the standard and the results were expressed in mg CE (catechin equivalents) per g.

2.4 Identification of phenolic compounds in the extract

FM-PRE and KM-PRE were filtered through a 0.2 μm syringe filter before injecting them into HPLC. 20 μL of the sample was injected into an autosampler and eluted through a reverse-phase C18 column YMC Triart (250 × 4.6 mm ID, S-5, 120 A, 5 μm, YMC Co. Ltd, Kyoto, Japan) with a gradient mobile phase consisting of A (water containing 0.1% formic acid) and B (methanol containing 0.1% formic acid) and the flow rate was set at 1 ml min^{-1}. A 30 min linear gradient was programmed. The gradient program was started with 98% of A and 2% of B at different time intervals. The phenolic compounds were identified using a diode array detector (Agilent Technologies, 1260 infinity, Singapore) by comparing the retention times with those of the standard phenolic compounds. The quantification of the major phenolics (ppm) was based on the area under the peak at a wavelength of 280 nm. The phenolic compounds in PREs were confirmed using direct infusion of PREs into a quadrupole ion trap (AB Sciex, Qtrap 5500, Singapore) equipped with an electrospray ionizer (ESI). The Qtrap was operated in negative and positive ion modes with curtain gas flow (CUR) at 30 psi, at an ion spray voltage of −4500 V and an ion source (TEM) temperature of 40 °C, with the ion source gas (GSI) at 30 psi. The syringe flow rate was set at 7 μL min^{-1}. Both MS and MS/MS data were acquired as individual phenolics for both FM-PRE and KM-PRE, which were further confirmed by matching their MS and MS/MS data with those of the standard phenolics.

2.5 Assessment of the anti-inflammatory effect of FM-PRE and KM-PRE on LPS induced inflammation in murine macrophage (RAW 264.7) cell line

2.5.1 Cell viability Assay. The mitochondrial reduction of MTT (03-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan was used to measure the cell viability. RAW 264.7 cells were seeded in DMEM supplemented with 10% Fetal Bovine Serum (FBS) and 1% PS at a density of 1×10^5 cells per well in a 96 well plate. Confluent cells were treated with 0.5 mg mL^{-1} and 1 mg mL^{-1} of FM-PRE and KM-PRE dissolved in DMSO (0.1%) as suggested in earlier reports.[23] Cells treated with 0.1% (v/v) DMSO were used as the

control. After 24 h, the supernatants were discarded and MTT (0.5 mg mL^{-1}) was added to each well and incubated for 4 h at 37 °C under dark conditions followed by the dissolution of insoluble formazan crystals in 100 μL DMSO. The absorbance of the resultant solution was measured at 570 nm using a microplate reader (NANO Quant-infinite M200 PRO, Tecan Life Science, Switzerland).

2.5.2 Prevention of LPS induced inflammation in macrophages. RAW 264.7 cells were seeded in DMEM supplemented with 10% FBS and 1% PS at a density of 1×10^5 cells per well in 96-well plates, followed by incubation for 24 h at 37 °C under 5% CO_2. Post confluence, cells were treated with 0.5 and 1.0 mg mL^{-1} of either KM-PRE or FM-PRE alone or in combination with LPS (1 μg mL^{-1}) for 24 h. Nitric oxide (NO) production in the supernatants was determined using the Griess reagent method.[23] The supernatants were also analyzed for TNF-α, IL-6, and IL-1β using ELISA kits as per the manufacturer's instructions.

2.6 *In vivo* experimentation

Male Swiss albino mice (7–8 weeks old; 18–22 g) were procured from Central Animal Facility (CAF) of National Institute of Pharmaceutical Education and Research (NIPER), S.A.S. Nagar, Punjab, India, and acclimatized for a week to the experimental conditions of CAF NIPER (maintained at 22 ± 2 °C; 55 ± 5% RH and a 12 h light/dark cycle). All the procedures used in this study were approved by the Institutional Animal Ethics Committee (IAEC) NIPER (IAEC 12/84) and were in accordance with the committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. Food and water were given *ad libitum* as per the treatment group. Initially, mice were randomized based on their body weights into two groups and fed a normal rodent pellet diet (NPD) or a high-fat diet (HFD). After two weeks of feeding, mice that showed ≥10% body weight gain from the initial weight (responders) on HFD feeding were selected and those that showed <10% weight gain (non-responders) were excluded from the study. Mice in the NPD group were divided into (i) NPD + vehicle, fed with NPD and vehicle; (ii) NPD + FM-PRE, fed with NPD and 50 mg of FM-PRE per kg of body weight and (iii) NPD + KM-PRE, fed with NPD and 50 mg of KM-PRE per kg of body weight. Similarly, HFD fed mice were divided into (i) HFD + vehicle, fed with HFD and vehicle (ii) HFD + FM-PRE, fed with HFD and 50 mg of FM-PRE per kg of body weight and (iii) HFD + KM-PRE, fed with HFD and 50 mg of KM-PRE per kg of body weight. The number of mice in each group was six and three mice were housed in each cage. FM-PRE and KM-PRE were freshly dissolved in a vehicle, which was 0.01% (v/v) tween 80 and were orally administered daily using gastric gavage as per the group description. The dose selection was based on the previously reported studies by several research groups.[24,25] Normal pellet diet (NPD, 3.79 kcal g^{-1}, 10% calories of food derived from fat, procured from Aashirvad Industries, Mohali, India) was used to prepare HFD (4.66 kcal g^{-1}, 60% calories of food derived from a fat source) as reported in our previous work.[26] The composition of HFD was as follows: powdered NPD (365 g kg^{-1}), lard (320 g kg^{-1}), milk casein (250 g kg^{-1}), vitamin and mineral mix (60 g kg^{-1}), DL-methionine (03 g kg^{-1}), yeast powder (01 g kg^{-1}), and sodium chloride (01 g kg^{-1}).

The duration of the experiment was 8 weeks. Body weights were measured every week and morphometric measurements were used to calculate their Lee's index ((weight$^{0.33}$ × 1000)/Naso-anal length) on the 6th day of the 8th week. On the day of sacrifice, blood and feces were collected in sterile containers. After 6 h of fasting, mice were euthanized by cervical dislocation followed by collection of the caecal content, visceral adipose (vWAT) and liver tissues. Half of the vWAT and liver tissues were snap-frozen and stored at −80 °C for ELISAs and gene expression studies while the other half of the tissues were stored in 10% neutral buffered formalin for histological analysis.

2.6.1 Oral glucose tolerance test and index of insulin resistance. On the 4th day of the 8th week, between 9:00 AM and 3:00 PM, the oral glucose tolerance test (OGTT) was performed on 6 h fasted mice. The fasting blood glucose concentration was measured and an oral dose of 2 g kg^{-1} of D-glucose was administered, followed by blood glucose measurements at 15, 30, 45, 60, 90, and 120 min using a Glucocard™ (Arkray Factory Inc., Shiga, Japan). The area under the curve (AUC) of blood glucose *versus* time graph was used for the interpretation of OGTT results. Different insulin resistance indices were calculated as previously described.[27]

2.6.2 Analysis of blood biochemical parameters. Serum triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL), and low density lipoprotein (LDL) were estimated using commercially available colorimetric assay kits as per the manufacturer's instructions (Accurex Biomedical Pvt. Ltd, Mumbai, India). The leptin, insulin, TNF-α, IL-1β, and LPS levels in serum were determined using the ELISA method as per the manufacturer's instructions.

2.6.3 Estimation of liver triglyceride. Approximately 50 mg of liver tissue was lyophilized followed by partitioning of the tissue in a chloroform : methanol (3 : 1) mixture and the extract was finally partitioned again with water to separate the chloroform layer. The chloroform layer was evaporated and the residue obtained was weighed (total hepatic lipid content) and dissolved in 2.0% Triton X 100 to estimate the triglyceride content using commercially available colorimetric assay kits as per the manufacturer's instructions (Accurex Biomedical Pvt. Ltd, Mumbai, India).[27]

2.6.4 Estimation of the liver TNF-α levels. Approximately 10–20 mg of liver tissue was homogenized in ice-cold PBS containing a cocktail of protease inhibitors followed by sonication (ice bath) and centrifugation (10 000 rpm; 10 min; 4 °C). The levels of TNF-α were determined in the supernatant using an ELISA kit as per the manufacturer's instructions. The levels were expressed as pmol mg^{-1} of protein. The total protein content in the homogenates were quantified using the Bradford method.

2.6.5 vWAT and liver histology. Small pieces of isolated vWAT and liver tissues were stored in 10% buffered formalin

for 48 h. The tissue was serially dehydrated and embedded in paraffin using ethanol as a dehydrating agent following xylene treatment for clearing the tissues. The sections of 10 μm thickness were stained using hematoxylin and eosin staining (H&E). Images were captured using a Leica microscope.

2.6.6 RNA extraction and qPCR. Total RNA was extracted from vWAT and liver using the Qiazol based RNA extraction method. Briefly, tissues were homogenized in Qiazol and centrifuged to remove debris. Chloroform based phase separation followed by RNA precipitation with isopropanol was used to isolate the total RNA. The integrity of RNA was tested on 1.2% agarose gel and quantified by Nanodrop at 260/280 nm. A Quantinova RT-synthesis kit (Qiagen, CA, USA) was used to synthesize cDNA from RNA. Changes in gene expression were determined by qPCR (Applied Biosystems 7500 Fast Real-Time PCR) based on SYBR green dye under the following conditions: 95 °C for 10 min, followed by 40 cycles of 95 °C and 60 °C for 1 min. The ΔΔCt method was used to analyze the qPCR data, and the values were expressed in terms of fold change relative to the control group.

2.6.7 Determination of *Firmicutes* and *Bacteroidetes* abundances and the short-chain fatty acid level in the caecal content. For the determination of *Firmicutes* and *Bacteroidetes* abundance, microbial DNA was isolated from the caecal content using a QIAmp DNA stool mini kit (Qiagen, Hilden, Germany), the integrity of the DNA was checked on 0.8% agarose gel and quantified using a UV spectrometer (Infinite® M200 Pro NanoQuant, Tecan, Switzerland). Finally, qPCR was performed to determine their abundances using previously validated primers[27] (Table 3). The short chain fatty acid (acetate, propionate and butyrate) levels were quantified using an Agilent 1260 infinity series chromatographic system (Agilent Technology, Singapore) equipped with an Agilent controller pump (model no. G1311C) and an autosampler unit (model no. G1329B) and separated using an anion exchange column (300 × 7.7 mm; 8 μm particle size). The standard SCFA mix (Sigma-Aldrich Co., St Louis, MO, USA) was used for quantification. The column was equilibrated and eluted with an isocratic flow of 0.1% formic acid in Milli-Q water at a rate of 0.6 mL min^{-1} at 60 °C for 30 min. A diode array detector (DAD; model no. G1315D) at 210 nm (peak width, 2.5 Hz and bandwidth, 2 nm) was used to detect individual fatty acids.[27]

2.7 Statistical analysis

Values were expressed as mean ± SEM. One way/repeated measure ANOVA followed by Tukey's *post hoc* test was used to compare different groups. GraphPad Prism 8 Software (GraphPad prism 8.0 Software Inc., La Jolla, CA, USA) was used to analyze the data. $P \leq 0.05$ was considered to be statistically significant.

3. Results

3.1 Extraction and characterization of PREs

Among the FM varieties, Co(Ra)14 and BB Royal market varieties showed nearly similar yields of polyphenols during methanolic extraction while the allimoyar variety showed a comparatively low yield (Table 1). Among the KM varieties, JK-65 and koraipatti varieties showed the same yield of polyphenol extracts while the varagu rice variety showed a lower yield. In general, the yield of the methanol extract of FM was higher than KM varieties (Table 1). The TPC of KM-JK-65 and KM-varagu rice varieties were higher than all the three tested varieties of FM, while the least TPC was found in the koraipatti variety of KM among all the tested varieties (Table 1). The TFC of KM-JK-65 and KM-varagu rice varieties were higher relative to all of the FM varieties tested while KM-koraipatti contained the least TFC among all. Similarly, the TTC was the highest in KM-JK-65 and KM-varagu rice varieties among all of the FM varieties tested, whereas KM-koraipatti had approximately similar TTC to FM-Co(Ra)14 and FM-BB royal varieties (Table 1). The FM-allimoyar local variety had the lowest TTC among all the varieties tested. On the basis of the above

Table 3 List of primers used in this study

	Forward 5′–3′	Reverse 5′–3′
ACOX1	GTGCAGCTCAGAGTCTGTCCAA	TACTGCTGCGTCTGAAAATCCA
β-*actin*	TGTTACCAACTGGGACGACA	GGGGTGTTGAAGGTCTCAAA
C/EBP α	CCAGTGACAATGACCGCCT	CGACCCTAAACCATCCTCCG
FASN	CTGCCTCTGGTGCTTGCT	GTCCGTGTACTCCTCCCCT
GCK	CGGATGGTGGATGAGAGCTC	CATTTCACAGGGCAGGGGAT
GLUT4	GATTCTGCTGCCCTTCTGTC	ATTGGACGCTCTCTCTCCAA
G6Pase	GTGCAGCTGAACGTCTGTCTGT	TCCGGAGGCTGGCATTGT
IR	TCCCCACCCTTTGAGTCTGA	GGGATCTTCGCTTTCGGGAT
IRS1	GGGAGATTCCAACACCAGCA	AGGCGTCCAGAGCTAGAAGA
IRS2	ATCAGGTATCTGGGGTGGAG	TGTGGCGCTTGGAATTGTGG
NFκB	GAGGTCTCTGGGGGTACCAT	TTGCGGAAGGATGTCTCCAC
PEPCK	GAACTGACAGACTCGCCCTATGT	GTTGCAGGCCCAGTTGTTG
PPAR-γ	GCCAAGGTGCTCCAGAAGAT	CTTTGTCAGCGACTGGGACT
SREBP-1c	GGAGCCATGGATTGCACATT	CACTGTCTTGGTTGTTGATGAGCTG
TNFα	AAGCCTGTAGCCCACGTCGTA	GGCACCACTAGTTGGTTGTCTTTG
Total bacteria	ACTCCTACGGGAGGCAGCAGT	ATTACCGCGGCTGCTGGC
BACT	ACGCTAGCTACAGGCTTAACA	ACGCTACTTGGCTGGTTCA
FIRM	GCGTGAGTGAAGAAGT	CTACGCTCCCTTTACAC

Table 1 Total phenolic, flavonoid and tannin contents in polyphenol-rich extracts of different varieties of finger millet and kodo millet

	Finger millet			Kodo millet		
	CO(Ra)14	Market variety (BB Royal)	Allimoyar local	JK-65	Varagu rice (BB Royal)	Koraipatti
Extract yield (%)	9.8 ± 0.1[a]	10.8 ± 0.3[a]	5.6 ± 0.9[b]	7.5 ± 1.0[d]	5.8 ± 0.1[e]	7.8 ± 0.1[d]
TPC (%GAE μg eq.)	5 ± 0.02[a]	5.45 ± 0.1[a]	3.2 ± 1.0[b]	9 ± 0.3[d]	7.16 ± 0.8[e]	2.15 ± 0.1[f]
TFC (%QE μg eq.)	3.6 ± 0.2[a]	3 ± 1.4[a]	2.3 ± 0.7[b]	24 ± 0.5[d]	18 ± 0.8[e]	1.58 ± 0.1[f]
TTC (%CE μg eq.)	9.5 ± 0.9[a]	10.8 ± 0.5[a]	6.7 ± 0.1[b]	13.8 ± 0.3[d]	12.5 ± 0.3[e]	9.78 ± 0.1[f]

GAE: Gallic acid equivalents, QE: quercetin equivalents, and CE: catechin equivalents. Values are expressed as mean of three replicates ± SEM. a, b, and c are significant relative to FM varieties whereas d, e, and f are significant relative to KM varieties. Different letters within the same test indicate significant differences at $p < 0.05$ by Tukey's test.

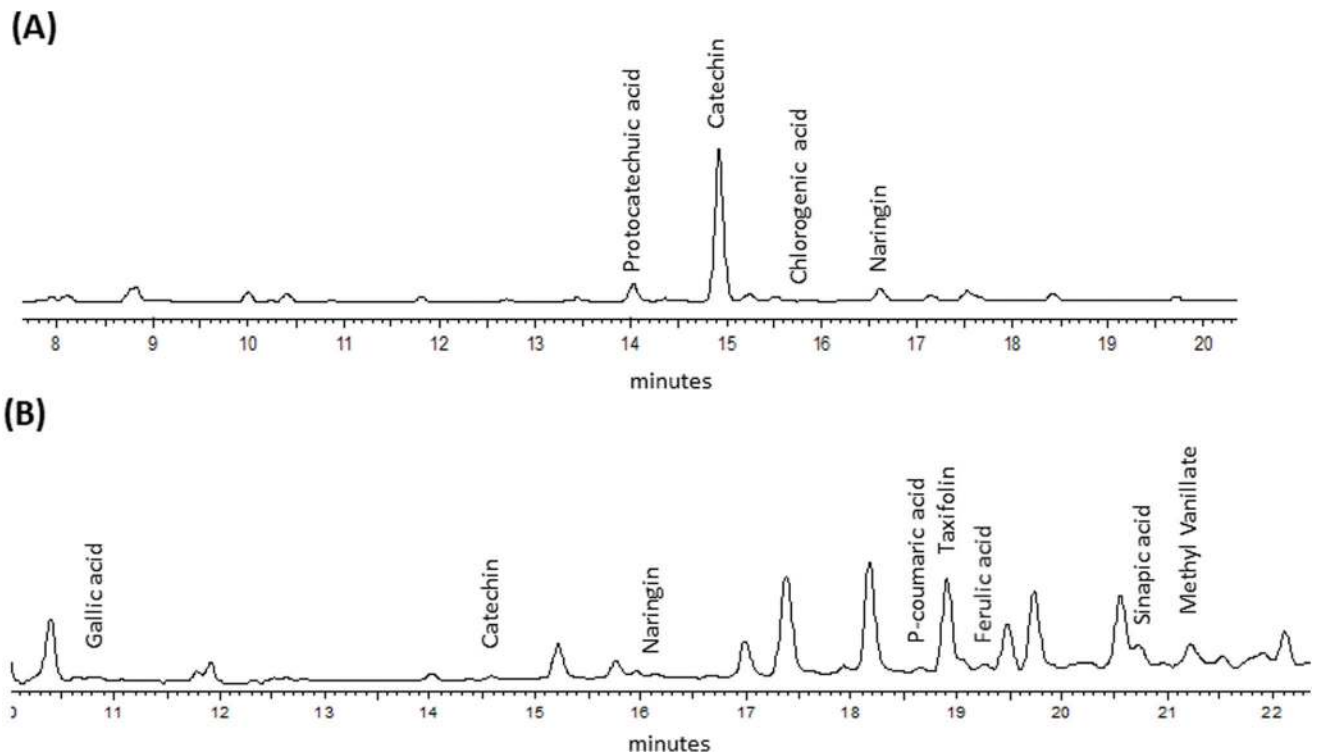

Fig. 1 HPLC-DAD chromatogram of major phenolic compounds. (A) Finger millet chromatogram peak identification was as follows: 1. protocatechuic acid, 2. catechin, 3. chlorogenic acid, and 4. naringin, (B) kodo millet chromatogram peak identification was as follows: 1. catechin, 2. naringin 3. *p*-coumaric acid 4. taxifolin 5. ferulic acid 6. sinapic acid and 7. methyl vanillate.

results, PREs from KM-JK-65 and FM Co(Ra)14 varieties were selected for further studies.

The phenolic profile and its identification on the basis of retention time are shown in representative chromatograms where the major compounds were aligned from the lowest to highest retention time (Fig. 1 and Table 2). In total, twelve phenolic compounds were identified in FM-PRE, which chiefly contained catechin (86.6%), vanillic acid (0.5%), chlorogenic acid (2.2%), and protocatechuic acid (5.6%) whereas taxifolin (98%), ferulic acid (0.19%) and naringin (0.1%) were the chief phenolics in KM-PRE (Table 2). Furthermore, based on ESI-MS (mass and MS/MS), protocatechuic acid, catechin, ferulic acid, and homovanillic acid were confirmed in FM-PRE, while taxifolin, gallic acid, protocatechuic acid, catechin, ferulic acid, and *p*-coumaric acid were confirmed in KM-PRE in the negative ion mode.

3.2 *In vitro* assessment of the anti-inflammatory effect of polyphenol-rich extracts against LPS induced inflammation in murine macrophage cell line (RAW 264.7)

MTT assay suggested that both FM-PRE and KM-PRE did not have any effect on cell viability at 0.5 and 1.0 mg mL^{-1} doses (Fig. 2A). Interestingly, LPS-induced NO production was prevented by FM-PRE and KM-PRE at 0.5 and 1 mg mL^{-1}. However, the activity of KM-PRE was more pronounced than FM-PRE (Fig. 2B). FM-PRE and KM-PRE at 1 mg mL^{-1} dose showed a greater reduction in NO production than at a dose of 0.5 mg mL^{-1} without compromising on cell viability (Fig. 2A

Table 2 Identification of phenolic compounds in FM-PREs and KM-PREs on the basis of retention time (t_R) (HPLC-DAD) and mass measurements (ESI-MS)

				Ion mode		MS/MS			Phenolics identified				Concentration (ppm) ± SEM		% abundance of phenolics	
									FM		KM					
Peak ID	Class of compound	Phenolic compound	Mass	+ve	−ve	+ve	−ve	t_R (min)	RT	Mass	RT	Mass	FM	KM	FM	KM
1	Hydroxybenzoic acid	Gallic acid	170	—	169	—	125	10.5	C	—	C	C	3.57 ± 0.5	0.98 ± 0.003	0.05	0.009
2		Vanillic acid	168	169	—	125, 93	—	17.0	C	—	C	—	32.01 ± 1.76	0.12 ± 0.004	0.5	0.001
3		Protocatechuic acid	154	—	153	—	109	13.7	C	C	C	C	343.4 ± 7.4	0.66 ± 0.24	5.6	0.006
4	Flavanols	Catechin	290.2					14.6	C	C	C	C	5281.7 ± 223.7	1.10 ± 0.04	86.6	0.01
5		Epicatechin	290	291	289	207, 165	245, 203	16.3	C	—	C	—	5.95 ± 0.05	—	0.09	0
6	Flavonols	Kaempferol	285	—	284	—	117, 93, 43	24.9	C	—	C	—	1.35 ± 0.46	1.10 ± 0.52	0.02	0.01
7		Quercetin	302	303	301	215	137, 122	23.4	C	—	C	—	12.76 ± 1.96	3.45 ± 0.02	0.20	0.03
8	Hydroxycinnamic acid	Ferulic acid	194	—	193	—	178, 133	19.3	C	C	C	C	17.95 ± 2.45	20.45 ± 0.51	0.29	0.19
9		*P*-Coumaric acid	164	—	163	—	118	19.2	C	—	C	C	2 ± 0.004	1.38 ± 0.008	0.03	0.01
10		Sinapic acid	224	225	223	206, 175	208, 164	19.1	C	—	C	—	—	7.9 ± 0.62	0.12	0.07
11	Flavanones	Homovanillic acid	182	—	181	—	137, 122	16.8	C	C	C	—	29.09 ± 8.41	4.94 ± 0.67	0.4	0.04
12		Naringin	580	581	579	273	459, 339, 313	19.6	C	—	C	—	218.57 ± 2.96	11.91 ± 0.079	3.58	0.11
13		Taxifolin	304	305	303	259, 153	125, 177, 285	18.8	C	—	C	C	12.22 ± 0.02	10 283 ± 21.5	0.2	98
14	Benzoic acid	Methyl vanillate	182	183	181	151, 124, 107	166	21	C	—	C	—	2.51 ± 1.01	4.74 ± 0.27	0.04	0.04
15	Ester (caffeic acid)	Chlorogenic acid	354	355	353	191	163	15.4	C	—	C	—	134.2 ± 1.46	5.91 ± 0.13	2.20	0.05

C – confirmed.

and B). Hence, 1 mg mL^{-1} concentration of both extracts was used to evaluate the inhibition of TNF-α, IL-1β, and IL-6 production by LPS treated macrophages. LPS treatment for 24 h resulted in the production of higher levels of IL-1β, IL-6, and TNF-α by the macrophages. This was prevented by FM-PRE and KM-PRE cotreatment with LPS in macrophages (Fig. 2C–E).

3.3 Kodo millet and finger millet polyphenol-rich extracts ameliorated HFD-induced weight gain, fat deposition, and gut bacterial dysbiosis

HFD fed mice gained more body weight than mice fed with NPD (Fig. 3A and B). FM-PREs and KM-PREs prevented the HFD-induced weight gain; however prevention in weight gain with KM-PRE was more potent than that with FM-PRE (Fig. 3A and B). The Lee's index, a marker of rodent visceral adiposity, was increased in HFD fed mice (Fig. 3C). Supplementation of FM-PRE and KM-PRE along with HFD prevented an increase in the Lee's index, although the difference was statistically significant only with KM-PRE (Fig. 3C). HFD fed mice showed a higher amount of vWAT deposition with larger size of adipocytes than NPD fed mice (Fig. 4A and C). Supplementation of KM-PRE and FM-PRE along with HFD prevented fat deposition and adipocyte hypertrophy, and KM-PRE conferred a better preventive effect than FM-PRE (Fig. 4A and C). An increase in the mRNA levels of *PPARγ* and *SREBP-1c* was observed in vWAT of HFD fed mice as compared to NPD fed mice (Fig. 4B). Co-administration of KM-PRE and FM-PRE along with HFD prevented the increase in *PPARγ* and *SREBP-1c*, with KM-PRE being more potent than FM-PRE (Fig. 4B).

As compared to NPD fed mice, HFD fed animals showed less amount of acetic acid, butyric acid and total SCFA in the caecal contents and a significant reduction in propionic acid production (Fig. 5A–D). In addition, the caecal gut bacteria of HFD fed animals had fewer *Bacteroidetes* and higher *Firmicutes* relative to their NPD counterparts (Fig. 5E). Furthermore, a decreased *Bacteroidetes* to *Firmicutes* ratio was observed in HFD fed mice. KM-PRE reversed the HFD induced alteration in the *Bacteroidetes* to *Firmicutes* ratio as well as the caecal propionic acid content whereas FM-PRE decreased the *Firmicutes* levels without altering the *Bacteroidetes* levels (Fig. 5A–E). Interestingly, both FM-PREs and KM-PREs increased the *Bacteroidetes* and decreased the *Firmicutes* levels in NPD fed mice (*per se* effect).

3.4 Supplementation of polyphenol rich extracts of FM and KM prevented the HFD-induced alterations in the serum lipid profile and systemic inflammation

HFD feeding increased the serum levels of TG as compared to NPD fed mice (p = 0.06). This increase was prevented by KM-PRE supplementation, whereas a decrease in serum TG was not significant with FM-PRE (p = 0.08) (Fig. 6A). There was no difference observed in the LDL, HDL, and TC levels among all the experimental groups (Fig. 6B–D). However, the atherogenic fraction (AF) of cholesterol was increased in HFD fed mice as compared to NPD fed mice. This increase was pre-

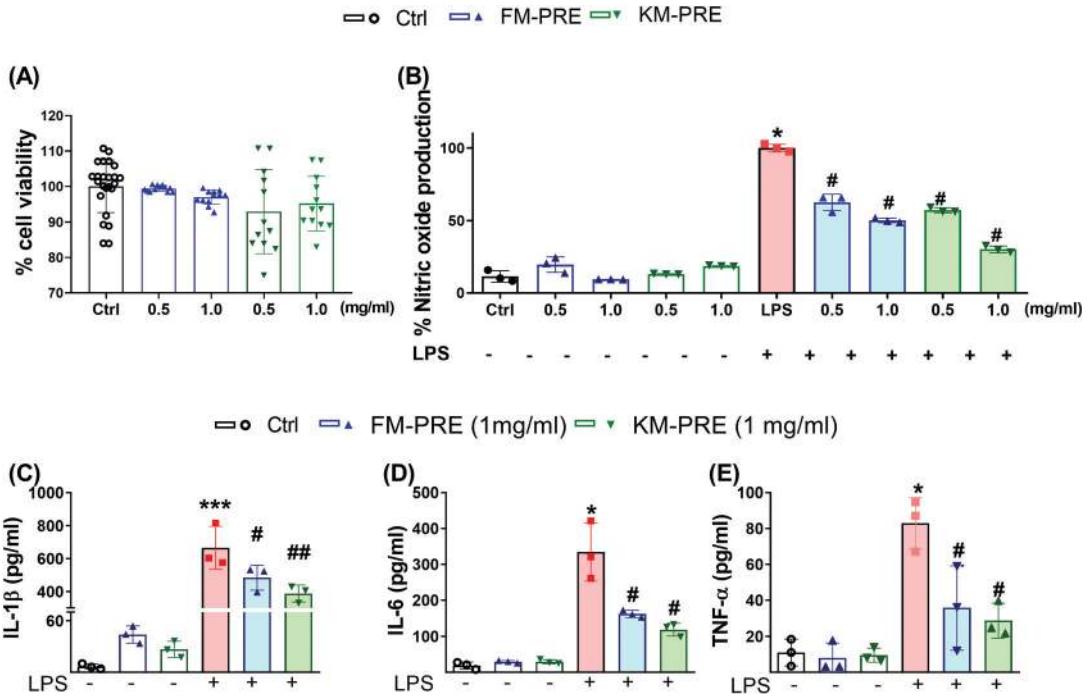

Fig. 2 Effect of KM-PRE and FM-PRE on (A) cell viability (MTT assay) of RAW 264.7 cells, (B) NO production, (C) IL-1β (D) IL-6 (E) TNF-α measured in the supernatants of LPS-stimulated RAW 264.7 cells. Data are presented as mean ± SEM; n = 15–25 in (A); n = 3 in (B–E) and evaluated by one-way ANOVA followed by Tukey's *post hoc* test ($p < 0.05$). * $P \leq 0.05$ relative to the control; *** $P \leq 0.001$ *vs.* control; # $P \leq 0.05$ relative to LPS; ## $P \leq 0.01$ *vs.* LPS. LPS – lipopolysaccharide, KM – kodo millet, and FM – finger millet.

vented by KM-PRE supplementation, while FM-PRE supplementation did not alter the AF of cholesterol as compared to HFD fed mice (Fig. 6E).

The serum leptin (marker of diet-induced visceral adiposity and metabolic complications) level was elevated in HFD fed mice as compared to NPD fed mice, which was reversed by FM-PRE and KM-PRE supplementation along with HFD (Fig. 6F). *Per se* supplementation of KM-PRE and FM-PRE did not affect the serum levels of leptin relative to NPD fed mice (Fig. 6F).

HFD feeding also increased the serum levels of LPS, TNF-α ($p = 0.08$), and IL-1β ($p = 0.06$) as compared to their respective levels in the NPD counterparts (Fig. 7A–C). Supplementation of KM-PRE curtailed the HFD induced increase in the serum LPS ($p = 0.08$), TNF-α, and IL-1β levels (Fig. 7A–C) whereas, FM-PRE reduced the serum level of IL-1β without affecting the TNF-α and LPS levels. In NPD fed mice, KM-PRE and FM-PRE supplementation did not alter the serum level of TNF-α, IL-1β, and LPS (Fig. 7A–C).

3.5 FM-PRE and KM-PRE supplementation prevented HFD-induced insulin resistance and hepatic steatosis

HFD fed mice showed reduced disposal of glucose load from circulation relative to NPD fed mice as observed in OGTT (Fig. 8A and B). Furthermore, HFD fed mice also exhibited increased serum insulin and HOMA-IR index (markers of insulin resistance) while the QUICKI index (insulin sensitivity index) was significantly reduced relative to NPD fed mice (Fig. 8C–E). Supplementation of KM-PRE and FM-PRE improved oral glucose clearance as compared to HFD fed mice (Fig. 8A and B). KM-PRE supplementation also improved the QUICKI index and prevented the increase in HOMA-IR while FM-PRE supplementation prevented the alterations in HOMA-IR (Fig. 8C–E). *Per se* supplementation of KM-PRE and FM-PRE along with NPD did not show any effect on these markers (Fig. 8C–E).

HFD fed mice exhibited increased hepatic steatosis markers with increased total hepatic lipids, hepatic TG and hepatic TNF-α accumulation (Fig. 9A–C). Consistent with the biochemical analysis, histological observations also suggested the increase in intrahepatic lipid accumulation in HFD fed mice relative to their NPD counter parts (Fig. 9D). Supplementation of KM-PRE and FM-PRE along with HFD significantly prevented the increase in the hepatic lipid, TG and TNF-α levels relative to HFD mice (Fig. 9A–C). Both KM-PRE and FM-PRE showed a reduction in hepatic lipid accumulation according to histological analysis; however, KM-PRE exhibited a more potent effect than FM-PRE (Fig. 9D).

HFD feeding enhanced the hepatic mRNA levels of *PPARγ* and *SREBP-1c* (key transcriptional factors regulating *de novo* lipid synthesis) as compared to NPD fed mice, whereas KM-PRE and FM-PRE supplementation along with HFD prevented the increase in their levels relative to HFD fed mice (Fig. 9E). Similarly, the mRNA levels of hepatic gluconeogenic genes, *i.e.*, *Glucokinase* and *G6Pase*, were up-regulated in HFD fed mice as compared to NPD fed mice, whereas KM-PRE and

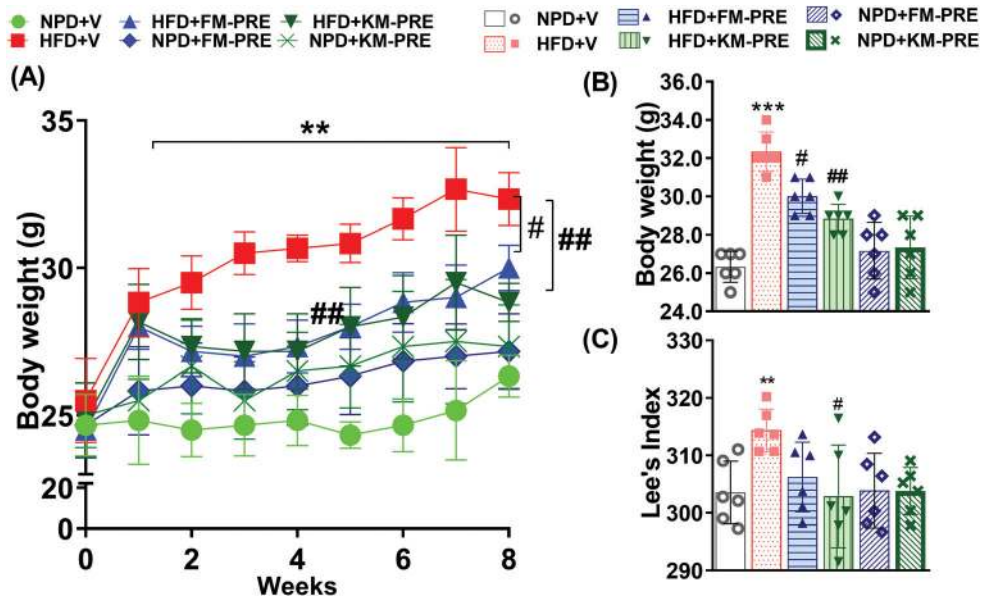

Fig. 3 Effect of KM-PRE and FM-PRE supplementation along with high-fat diet on (A) body weight progression, (B) average body weight, (C) Lee's index. Values are expressed as mean ± SEM; n = 6 and analyzed by RM ANOVA (A)/one-way ANOVA (B and C) followed by Tukey's *post hoc* test. **$p \leq 0.01$ *vs.* NPD + V; *** $P \leq 0.001$ *vs.* NPD + V; # $p \leq 0.05$ *vs.* HFD + V; ## $P \leq 0.01$ *vs.* HFD + V. NPD-V, normal pellet diet with vehicle; HFD + V, high-fat diet with vehicle; HFD + KM-PRE, high-fat diet with KM-PRE; HFD + FM-PRE, high-fat diet with FM-PRE; NPD + KM-PRE, normal pellet diet with KM-PRE; and NPD + FM-PRE, normal pellet diet with FM-PRE.

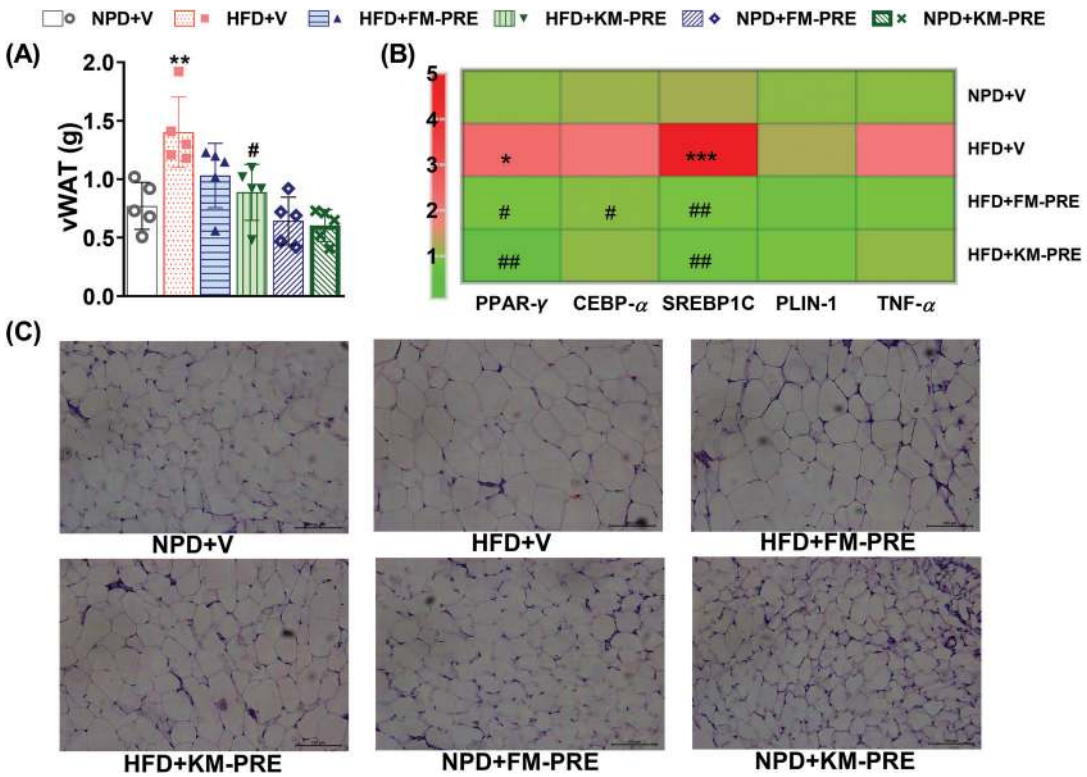

Fig. 4 Effect of KM-PRE and FM-PRE supplementation along with high-fat diet on (A) vWAT weight, (B) gene expression profile in adipose tissue, (C) representative image of H&E stained visceral adipose tissue (20× objective magnification; scale bar-100 μm). Values are expressed as mean ± SEM; n = 4–5 and analyzed by one-way ANOVA followed by Tukey's *post hoc* test. *$p \leq 0.05$ *vs.* NPD + V; **$p \leq 0.01$ *vs.* NPD + V; *** $P \leq 0.001$ *vs.* NPD + V; # $p \leq 0.05$ *vs.* HFD + V; ## $P \leq 0.01$ *vs.* HFD + V. NPD-V, normal pellet diet with vehicle; HFD + V, high-fat diet with vehicle; HFD + KM-PRE, high-fat diet with KM-PRE; HFD + FM-PRE, high-fat diet with FM-PRE; NPD + KM-PRE, normal pellet diet with KM-PRE; and NPD + FM-PRE, normal pellet diet with FM-PRE.

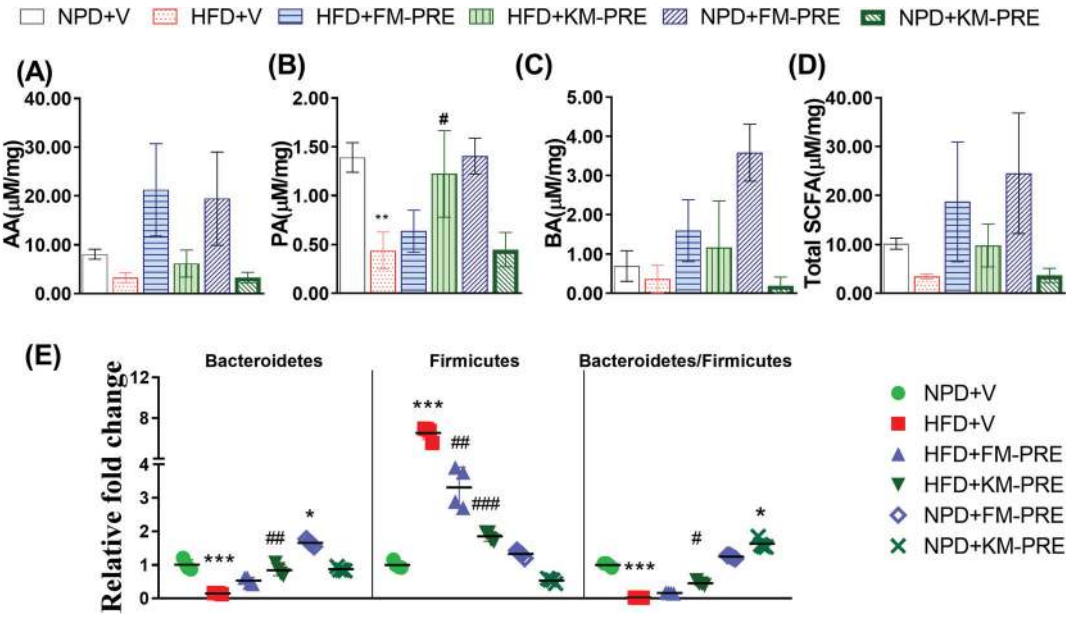

Fig. 5 Effect of KM-PRE and FM-PRE supplementation on relative bacterial abundances of (A) acetate (B) propionate (C) butyrate (D) total short-chain fatty acid levels (E) relative bacterial abundances. Values are expressed as mean ± SEM; n = 4–6 and analyzed by one-way ANOVA followed by Tukey's *post hoc* test. *$p \leq 0.05$ *vs.* NPD + V; **$p \leq 0.01$ *vs.* NPD + V; *** $P \leq 0.001$ *vs.* NPD + V; # $p \leq 0.05$ *vs.* HFD + V; ## $P \leq 0.01$ *vs.* HFD + V; ### $p \leq 0.001$ *vs.* HFD + V. NPD-V, normal pellet diet with vehicle; HFD + V, high-fat diet with vehicle; HFD + KM-PRE, high-fat diet with KM-PRE; HFD + FM-PRE, high-fat diet with FM-PRE; NPD + KM-PRE, normal pellet diet with KM-PRE; and NPD + FM-PRE, normal pellet diet with FM-PRE. C_T values were normalized against the genus-specific total bacteria sequence primer. The expression was expressed as a positive fold change relative to the control group.

FM-PRE supplementation along with HFD prevented their increase (Fig. 9E), while the expression of *PEPCK* was not altered in any of the groups. The expression of *TNF-α* was increased in HFD fed mice compared to NPD fed mice (Fig. 9E). KM-PRE and FM-PRE supplementation prevented this increase (Fig. 9E). The expression level of the insulin receptor was decreased in the livers of HFD fed animals as compared to NPD fed mice, and this decrease was prevented by both FM-PRE and KM-PRE (Fig. 9E).

4. Discussion

Chronic low grade inflammation underlying obesity is a primary contributing factor leading to multiple comorbidities such as insulin resistance, type II diabetes, and hepatosteatosis.[28,29] The recent alarming increase in the incidence of obesity points towards the need for techniques, tools, treatment, and preventive options to control this burden. In this regard, the search for novel functional foods and new dietary habits that could be able to treat or prevent obesity has increased multiple fold.[30] An increase in the consumption of whole grain cereals is an important alteration in daily food habits and is reported to show protective effects on the metabolic profile of the body. Health benefits offered by these cereals are majorly based on but not restricted to their polyphenol and dietary fiber contents.[13,14,30] Millets are suggested to have better nutritional quality as compared to major cereals in terms of phenolic acids, dietary fibers and antioxidant properties.[31] FM shows a higher free radical quenching activity than wheat and rice, the highest consumed cereals in the world.[20] The consumption of food products from FM also lowered TG and C-reactive protein as compared to rice products.[32] FM has also shown multiple health benefits including glucose-lowering properties, wound healing properties, anti-obesity properties, *etc*.[33,34] However, there are very limited reports on the role of polyphenols from millet especially those from FM and KM in protecting HFD induced obesity and associated complications. Our data showed higher phenolics in KM-PRE than in FM-PRE which is in agreement with the previous report[35] where KM has been shown to have the highest amount of both soluble and insoluble polyphenols as compared to the FM, foxtail millets, proso millets, pearl millets and little millets.[36] In the present study, FM-PRE and KM-PRE showed the presence of flavonoids as the major phenolics. HPLC and mass spectrometry data showed that KM-PRE contains nearly 98% taxifolin (dihydroquercetin) and FM-PRE contains 86.6% catechin and 5.6% protocatechuic acid as the major phenolics. We observed that both KM-PRE and FM-PRE reduced LPS induced NO production in the RAW 264.7 macrophage cells at a dose of 1 mg mL^{-1}. However, the anti-inflammatory effect of KM-PRE was relatively higher than that of FM-PRE. Moreover, KM-PRE also prevented HFD induced production of TNF-α, IL-6, and IL-1β more significantly than FM-PRE, when co-treated with LPS in the RAW 264.7 macrophage cells which is in agreement with the pre-

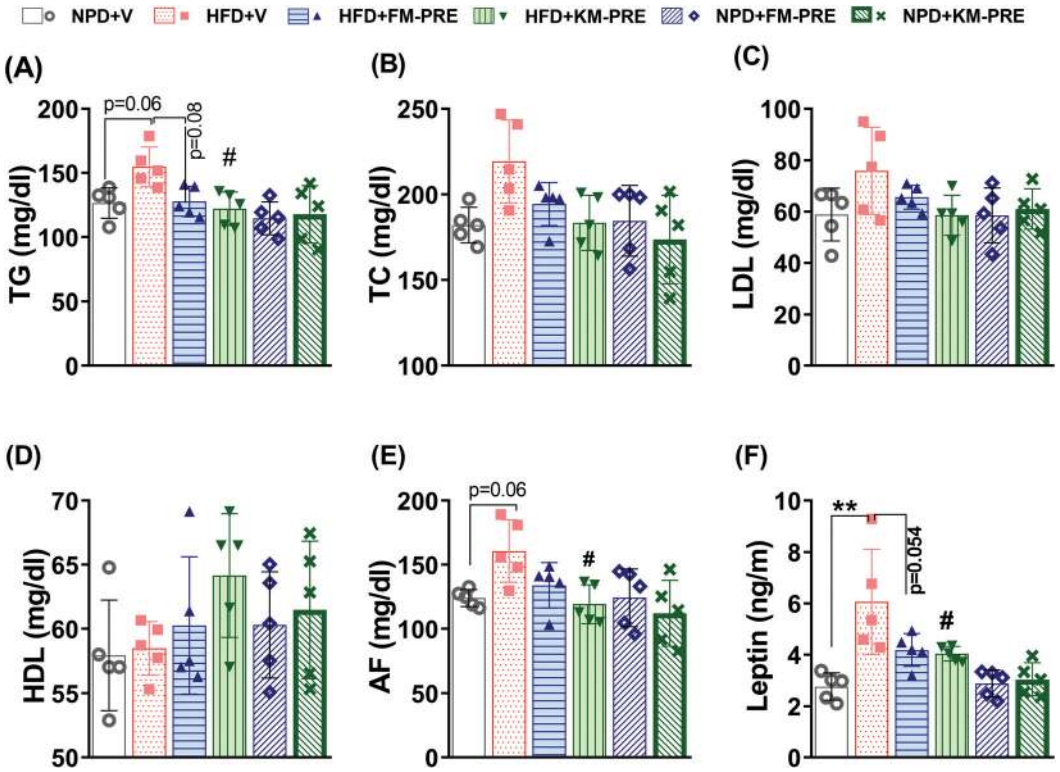

Fig. 6 Effect of KM-PRE and FM-PRE supplementation on (A) serum triglycerides, (B) serum total cholesterol, (C) serum low-density lipoprotein, (D) serum high-density lipoprotein, (E) serum atherogenic fraction, and (F) serum leptin. values are expressed as mean ± SEM; n = 5 and analyzed by one-way ANOVA followed by Tukey's *post hoc* test; **$p \leq 0.01$ *vs.* NPD + V; # $p \leq 0.05$ *vs.* HFD + V; individual comparative p values are expressed if slightly higher than 0.05. NPD-V, normal pellet diet with vehicle; HFD + V, high-fat diet with vehicle; HFD + KM-PRE, high-fat diet with KM-PRE; HFD + FM-PRE, high-fat diet with FM-PRE; NPD + KM-PRE, normal pellet diet with KM-PRE; and NPD + FM-PRE, normal pellet diet with FM-PRE.

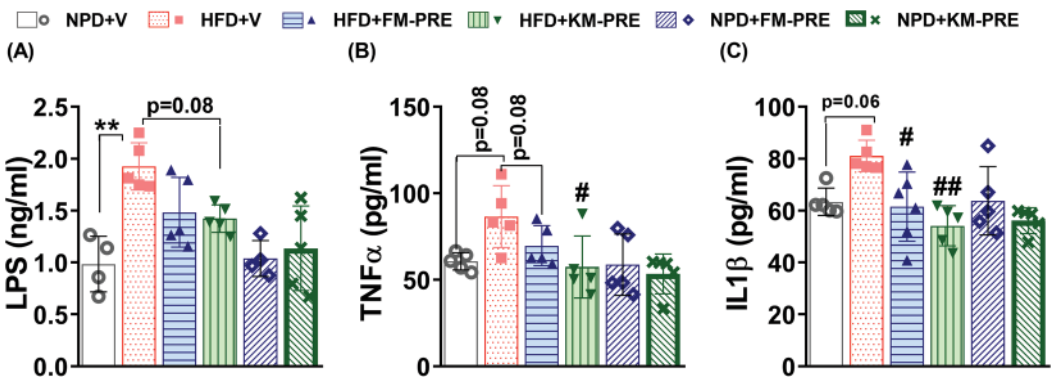

Fig. 7 Effect of KM-PRE and FM-PRE supplementation on (A) serum LPS, (B) serum TNF-α, (C) serum IL-1β. Values are expressed as mean ± SEM; n = 4–6 and analyzed by one-way ANOVA followed by Tukey's *post hoc* test. **$p \leq 0.01$ *vs.* NPD + V; # $p \leq 0.05$ *vs.* HFD + V; ## $P \leq 0.01$ *vs.* HFD + V. Individual comparative p values are expressed if slightly higher than 0.05. NPD-V, normal pellet diet with vehicle; HFD + V, high-fat diet with vehicle; HFD + KM-PRE, high-fat diet with KM-PRE; HFD + FM-PRE, high-fat diet with FM-PRE; NPD + KM-PRE, normal pellet diet with KM-PRE; and NPD + FM-PRE, normal pellet diet with FM-PRE.

vious literature which has reported that KM has shown high antioxidant activity compared to FM in terms of free radical quenching activity in DPPH assay as measured by electron spin resonance, which is attributed to the higher amount of TPC in KM.[19,20] Since insulin resistance, hepatosteatosis, and other metabolic abnormalities are closely linked to low-grade systemic inflammation, we hypothesized that chronic supplementation of these extracts could curtail the chronic low-grade inflammation underlying HFD-induced obesity. To this end, our *in vivo* studies suggested that KM-PRE and FM-PRE prevented HFD-induced weight gain and vWAT deposition, although the effect of KM-PRE was more significant. These

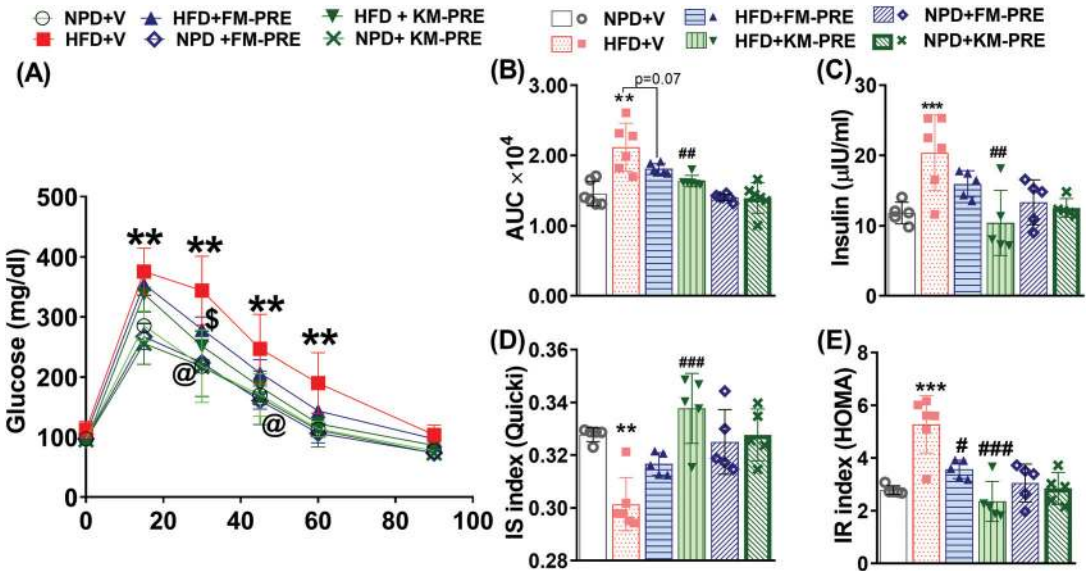

Fig. 8 Effect of KM-PRE and FM-PRE supplementation on (A) oral glucose tolerance test (B) AUC between blood glucose and time graph, (C) serum fasting insulin, (D) Quicki index, and (E) HOMA IR. For OGTT values are expressed as mean ± SEM; n = 5 and analyzed by RM ANOVA followed by Tukey's *post hoc* test. **$p \leq 0.01$ NPD + V *vs.* HFD + V; α $p \leq 0.01$ HFD + V *vs.* HFD + KM-PRE; $ $p \leq 0.01$ HFD + V *vs.* HFD + KM-PRE. For B–E, values were expressed as mean ± SEM; n = 5 and analyzed by one-way ANOVA followed by Tukey's *post hoc* test. **$p \leq 0.01$ *vs.* NPD + V; *** $P \leq 0.001$ *vs.* NPD + V; # $p \leq 0.05$ *vs.* HFD + V; ## $P \leq 0.01$ *vs.* HFD + V; ### $p \leq 0.001$ *vs.* HFD + V. NPD-V, normal pellet diet with vehicle; HFD + V, high-fat diet with vehicle; HFD + KM-PRE, high-fat diet with KM-PRE; HFD + FM-PRE, high-fat diet with FM-PRE; NPD + KM-PRE, normal pellet diet with KM-PRE; and NPD + FM-PRE, normal pellet diet with FM-PRE.

results support our previous finding that KM and FM bran feeding prevented HFD-induced metabolic alterations,[6,9] which may be due to the presence of polyphenols besides dietary fiber in the bran. In line with the *in vitro* study of macrophages, the *in vivo* study also indicated that KM-PRE exhibited a better anti-inflammatory effect than FM-PRE in terms of preventing the increased serum levels of TNFα, IL-1β and LPS. Furthermore, systemic insulin resistance (higher serum insulin levels, diminished insulin sensitivity indices, and impaired glucose tolerance) as a result of HFD feeding was prevented by FM-PRE and KM-PRE supplementation. However, the effects of KM-PRE were better than those of FM-PRE.

The gut microbiota plays an important role related to obesity and associated inflammatory disorders.[6,9,30,37] In this study, the caecal contents showed a higher *Bacteroidetes* to *Firmicutes* ratio with KM-PRE, which is considered to be an indication of weight loss.[38] Furthermore, KM-PRE fed mice had low levels of LPS in the systemic circulation, which otherwise was high in HFD fed mice. LPS in the gut is produced from Gram negative bacteria especially the pathogenic ones due to dysbiosis in the gut microflora and translocates into the systemic circulation due to dampened gut barrier function in obese animals and humans.[39] This systemic LPS has been proved to cause low grade inflammation in obesity and related comorbidities.[40] KM-PRE prevented these deleterious changes thereby protecting against the loss of gut barrier function due to HFD feeding. Dietary phenols exist as glycoside conjugates, which upon gut bacterial metabolism gets converted into bioactive aglycones and free sugar might be used by gut bacteria to promote SCFAs.[37] In the present study, the HFD induced decrease in propionic acid was reversed by KM-PRE, which is an indication of improved gut bacterial dysbiosis. Certain polyphenols or polyphenol-rich extracts have been shown to stimulate *Bifidobacteria* and *Lactobacilli* while others have been shown to stimulate *Akkermansia muciniphila*, which are the key microbial players in HFD-induced obesity.[41,42] In this regard, both FM-PRE and KM-PRE might be useful as gut microbial stimulants, which needs further investigations.

The gene expression study in vWAT indicated decreased *PPARγ* and *SREBP-1c* expression in mice fed with FM-PRE and KM-PRE with KM-PRE being more potent. This is due to the prevention of hypertrophy and hyperplasia of adipocytes, as evident from histology, that causes higher expression of *PPARγ*, *C/EBP-α* and *SREBP-1c*, the markers of diet induced obesity.[43] Our results are in congruence with several other studies which have shown that polyphenols such as quercetin, coumaric acid, chlorogenic acid, catechin, resveratrol, and curcumin are known to downregulate *PPARγ* expression and increase insulin sensitivity.[44–46] Higher expression of *SREBP-1c* has been implicated in higher hepatic lipid accumulation.[47] Reversal of HFD-induced increase in liver *SREBP-1c* expression together with reduction of liver lipid in biochemical analysis by KM-PRE is suggestive of not only its beneficial effects on hepatic steatosis but also an improved insulin sensitivity. This observation is further strengthened by reversal of the HFD-induced *glucokinase* and *G6Pase* levels in the liver as well as the HOMA-IR and QUICKI index by KM-PRE. FM-PRE has shown its effect only in

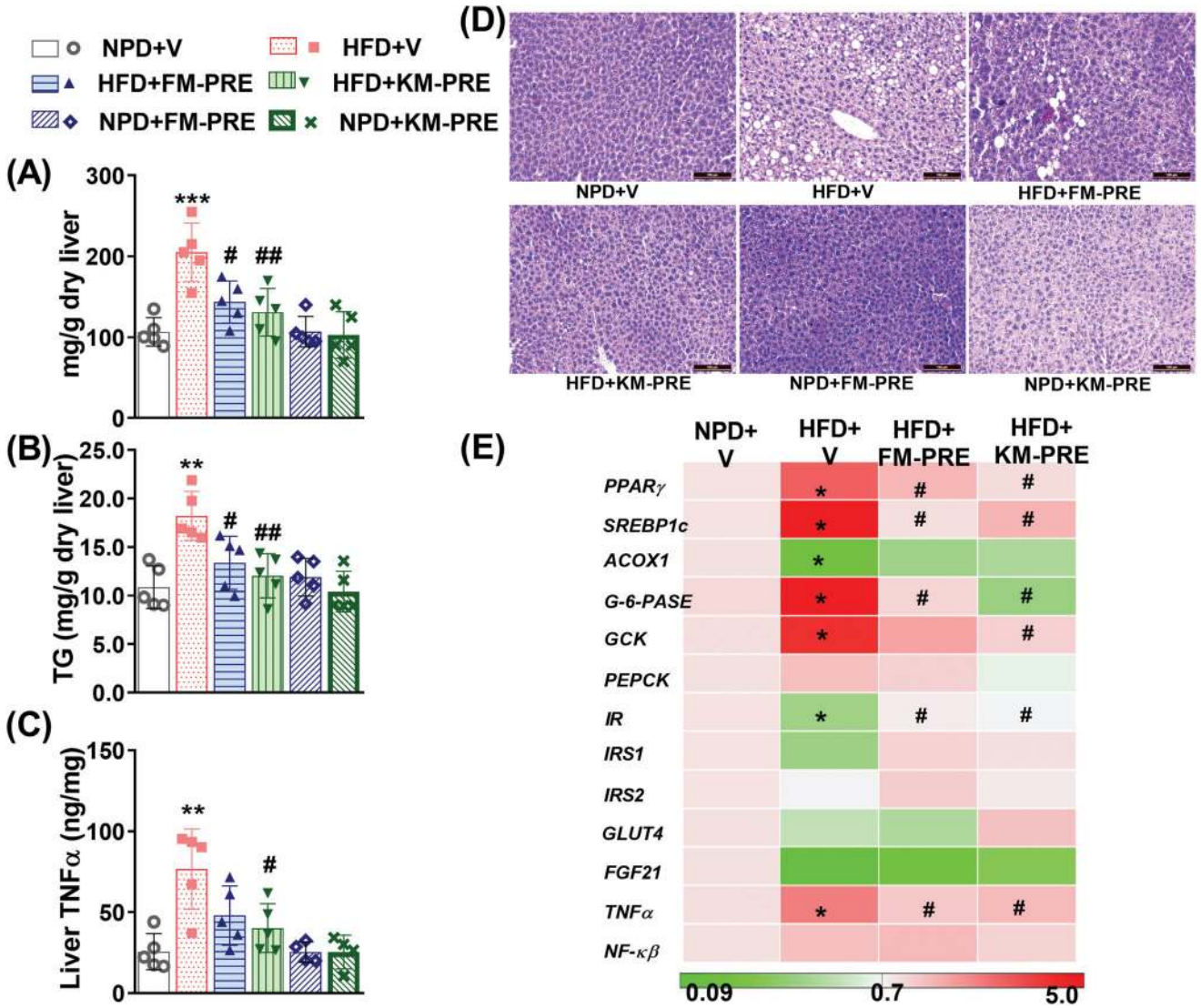

Fig. 9 Effect of KM-PRE and FM-PRE supplementation on (A) liver lipids, (B) liver TG, (C) liver TNF-α, (D) representative photomicrographs of H&E stained liver sections (20× objective magnification; scale bar-100 μm), and (E) RT-PCR expression in liver tissues. Values are expressed as mean ± SEM; n = 5 and analyzed by one-way ANOVA followed by Tukey's *post hoc* test. *$p \leq 0.05$ *vs.* NPD + V; **$p \leq 0.01$ *vs.* NPD + V; *** $P \leq 0.001$ *vs.* NPD + V; # $p \leq 0.05$ *vs.* HFD + V; ## $P \leq 0.01$ *vs.* HFD + V. NPD-V, normal pellet diet with vehicle; HFD + V, high-fat diet with vehicle; HFD + KM-PRE, high-fat diet with KM-PRE; HFD + FM-PRE, high-fat diet with FM-PRE; NPD + KM-PRE, normal pellet diet with KM-PRE; and NPD + FM-PRE, normal pellet diet with FM-PRE.

reversal of liver lipids, liver TG, *SREBP-1c*, *G6pase* and HOMA-IR index without showing any effect on *glucokinase* and QUICKI index.

As the polyphenols do not reach the target tissues at the optimum levels, direct scavenging of free radicals may not be the reason for the beneficial effects *in vivo* but instead, the nuclear transcription factor pathway, lipid metabolic pathway, and immunomodulatory pathway including TNFα, IL-1β, and IL-6 might be regulated through intra- and inter-cellular signaling.[48] In this study too, it is anticipated that the protective effects of FM-PRE and KM-PRE might involve any of these mechanisms as we could observe prevention of deleterious alterations in these pathways which otherwise are caused due to HFD feeding. The existing literature indicated that taxifolin confers a potent effect in combating metabolic complications than other flavonoids like catechin.[49–52] Although this study portrayed FM-PRE and KM-PRE to mitigate HFD-induced alterations, the detailed molecular mechanisms for the anti-obesity effects such as gut hormone regulation, immune regulation, role of specific gut microbiota and metabolites of administered polyphenols need to be elucidated.

In conclusion, the present study highlighted the anti-obesity effects of KM-PREs and FM-PREs in terms of preventing HFD-induced weight gain, insulin resistance, hepatic steatosis, and systemic inflammation (Graphical abstract). However, the magnitude of impeding inflammation and HFD-

induced alterations by KM-PRE was more pronounced than FM-PRE. These experimental findings would help in designing clinical investigations with these PREs for the management of obesity and comorbidities.

Abbreviations

BMI	Body mass index
CE	Catechin equivalent
FM	Finger millet
HFD	High fat diet
HPLC	High performance liquid chromatography
GAE	Gallic acid equivalent
KM	Kodo millet
LDL	Low-density lipoproteins
LPS	Lipopolysaccharide
NPD	Normal pellet diet
P/S	Penicillin/streptomycin
PRE	Polyphenol rich extract
QE	Quercetin equivalent
TFC	Total flavonoid content
TPC	Total phenolic content
TTC	Total tannin content
vWAT	Visceral adipose tissue

Author contributions

PK, RM, MB and KKK designed the experiments. PK, RM, and PM carried out experiments. PK, RM, JS, KKK, and MB carried out HPLC data analysis. RM and RB contributed to macrophage studies. KKK, MB, and KP reviewed and edited the paper.

KKK Lead contact.

Conflicts of interest

Authors declare no competing financial interest.

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