



## Original Article

# Anti-obesity Effects of SCOBY Jackfruit Beverages and Their Influence on Gut Microbiota



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## Abstract

**Background and objectives:** Changes in eating habits and a sedentary lifestyle have shifted the primary role of food as an energy source to nutritious food for maintaining good health. A new functional jackfruit beverage was produced using a selected symbiotic culture of bacteria and yeast (SCOBY) with the aim of developing a cost-effective anti-obesity therapy as a preventive measure.

**Methods:** A total of five groups of the Institute of Cancer Research mice consisting of a normal control, positive control, negative control, SCOBY jackfruit pulp, and jackfruit leaves treated mice were used to examine the anti-obesity efficacy of SCOBY jackfruit beverages. An analysis on the gut microbiota, quantitative polymerase chain reaction gene expression, short chain fatty acids, and blood composition profile was also investigated.

**Results:** High-fat diet-fed obese mice treated with SCOBY jackfruit beverages showed great improvement in the weight management control and significant body weight loss (18.5–20.2%) compared to a commercial anti-obesity drug, Orlistat (11.3%). There were no adverse effects on the blood composition profile and inflammation symptoms observed in the treated obese mice. The expression of the genes relating to glucose transport, lipid biosynthesis, inflammatory cytokines, and chemokines in the adipose tissues was significantly downregulated following the SCOBY jackfruit beverages diet interventions ( $P < 0.05$ ). The analysis of the 16S rRNA sequencing on the mice fecal samples

revealed that SCOBY jackfruit beverages had altered the gut microbiota composition with the enhanced growth of beneficial gut microbes in those treated mice relative to all control groups.

**Conclusions:** The findings in this study implied that SCOBY jackfruit beverages were potentially useful as a new therapeutic strategy for weight management control.

**Keywords:** Anti-obesity; jackfruit; microbiota; short chain fatty acids.

**Abbreviations:** SCOBY, symbiotic culture of bacteria and yeast; MARDI, Malaysian Agricultural Research and Development Institute; qPCR, quantitative polymerase chain reaction; SCFAs, short chain fatty acids; JP, jackfruit pulp; JL, jackfruit leaves; HFD, high-fat diet; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; TP, total protein; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; Actb, Actin-b; Hprt, hypoxanthine phosphoribosyl transferase-encoding gene; Slc2a4, Glucose transporter 4; SREBP-1, sterol regulatory element binding protein 1; Adipor 1, adiponectin receptor 1; NOS2, nitric oxide synthase 2; TNF- $\alpha$ , tumor necrosis factor alpha; TGF- $\beta$ 1, transforming growth factor  $\beta$ 1; MMP2, matrix metalloproteinase 2; IL6, interleukin 6; CCL2, C-C motif chemokine ligand 2; OTUs, operational taxonomic units.

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## Introduction

In recent decades, the prevalence of an overweight and obese populace has increased exponentially worldwide and contributed to substantial morbidity and mortality. The latest statistics recorded Malaysia as the ‘fattest’ country in Southeast Asia, whereby overweight (38.5%) and obese (13.3%) people comprised approxi-

mately half of its 30 million populace,<sup>1</sup> thus indicating an alarming increase in the country's obesity rate. This phenomenon has caused a major health concern and its incalculable medical costs have become an economic burden on the government. Additionally, obesity is the primary causative factor for various metabolic disorders and chronic diseases, including diabetes, hypertension, osteoarthritis, inflammation, cardiovascular disease, *etc.*<sup>2</sup> Globally, there is an increase in the trend of health concerned consumers who are looking for a natural therapeutic agent with minimum or zero side effects for the prevention and treatment of many illnesses related to obesity to replace the currently available medication.

In addition, recently, there have been many studies focusing on the interaction between gut microbiota with obesity and multiple obesity-associated health consequences, including Type 2 diabetes, cardiovascular disease, and several cancers, which contribute to the enormous economic burden.<sup>3-5</sup> Gut microbiota has been identified as an integral mediator in the pathophysiology of obesity and related metabolic disorders. This is due to the alteration of the gut microbiota, so the immune system is affected, thus resulting in overweight people where the modification of host obesity is linked to gut microbiota which is involved in the changes in the energy extraction, intestinal permeability, and systemic inflammation.<sup>6</sup> Past studies have also revealed the presence of some key gut microbial metabolites, such as short chain fatty acids (SCFAs). These were identified as the potential therapeutic targets for diet-induced obesity, as these modulated the effects of the diet, as well as regulated the host metabolism and appetite.<sup>7-9</sup> As a consequence, the status of obesity has a great influence on the degree of alteration of the microbial microbiota colonized in the gastrointestinal tract which in turn affects the capability of the production of obesity-suppressing SCFAs. The SCFAs and their receptors pertain to the efficacy of the diet interventions and gut microbiota manipulations in the management of obesity and energy metabolic syndrome. Furthermore, exposure to the gut bacterial components has implications for the pathogenesis of obesity *via* the induction of the low-grade inflammation of adipose tissue and modifications of the gut microbiota. Therefore, dysbiosis of the gut microbiota may cause an implication in the multitude of metabolic alterations.

Jackfruit (*Artocarpus heterophyllus* L.), one of the high-yielding fruit crops, is widely grown in Asia. The leaves of the jackfruit are known to have valuable pharmacological properties, such as anti-diabetic, anti-inflammatory, antibacterial, and many other medicinal attributes, which have been traditionally used in folk medicine.<sup>10</sup> Jackfruit is rich in phytonutrients, including phenolic compounds (flavonoids), vitamins (A, C, riboflavin, and thiamine), minerals (potassium, iron, zinc, and niacin), and other nutrients.<sup>11</sup> It is rich with a variety of phytochemicals that have made this fruit a suitable substrate for developing functional products to be utilized in food and nutraceutical applications.

Microbial fermentation serves to be one of the food processing strategies that have been traditionally used for improving food functional and nutritive values. Many scientific findings have reported that fermented foods are healthier and palatable with a better aroma and taste, as a consequence of the microbial work that is capable of hydrolyzing macronutrients into easily digestible and bioavailability phytonutrients.<sup>12</sup> A controlled biofermentation process has also been developed in the Malaysian Agricultural Research and Development Institute (MARDI) to produce fermented jackfruit phytonutrient enriched beverages using selected Kombucha consortium strains that consist of a pure symbiotic culture of yeast and bacteria (SCOBY). Hence, the resulting SCOBY jackfruit beverages have been found to have enhanced nutritional values, as they contain health beneficial organic compounds and secondary metabolites in addition to their organoleptic and functional properties due to the microbial activity.

Therefore, the ultimate objective of this work was to study the efficacy of SCOBY jackfruit beverages toward developing a natural anti-obesity therapeutic agent for obesity prevention and control measures. The effect of the SCOBY jackfruit beverages consumption on the gut microbiota system was also investigated.

## Materials and methods

### Preparation of SCOBY jackfruit beverages

Jackfruit (*Artocarpus heterophyllus* L.) leaves and pulp were purchased from a local jackfruit plantation in Lanchang, Pahang, Malaysia. The jackfruit leaves and pulp were used as substrates for the production of fermented jackfruit pulp (JP) and leaves (JL), respectively. The fermentation process was conducted according to the procedure as described in Sabidi *et al.*<sup>13</sup> The substrate was inoculated with two types of microorganisms: a) yeast (*Dekkera sp.*) and b) acetic acid bacteria (*Komagataibacter sp.*) selected from MARDI's Collection of Functional Food Culture (CFFC). After a week of the fermentation process, the supernatant was collected after centrifuging at 10,000 rpm for 10 m to remove any microbes and substrate residue, and the products were named as SCOBY jackfruit beverages (JP and JL), which were produced from the substrate of the jackfruit pulp and leaves, respectively.

### *In vivo* anti-obesity study on high-fat diet-fed obese mice

All Institute of Cancer Research (ICR) mice were placed in the MARDI and were acclimatized for two weeks in cages at  $25 \pm 2^\circ\text{C}$  in a 12 h light/dark condition. The mice were provided with standard sawdust bedding, distilled water, and commercial pellets (Gold Coin, Malaysia) before proceeding to a high-fat diet treatment.

A total of 30 ICR mice were used in the anti-obesity study and divided into five groups: a) normal diet; b) high-fat diet, HFD, C(-); c) HFD-Orlistat, C(+); d) HFD-fermented jackfruit pulp, JP; e) HFD-fermented jackfruit leaves, JL. A semi-pure high-fat rodent diet (34.5% fat; D12492) was purchased from Specialty Feeds, Australia and used to induce obesity. All mice groups except for the normal diet mice group, were fed with HFD for three months to make them obese (at least 20% body weight higher than normal diet mice) before being treated with Orlistat (10 mg/kg), JP (2 mL/kg), and JL (2 mL/kg). The dose of 2 mL was chosen based on the recommendation of ~20 mL of the daily human dose consumption of a 60 kg body weight. The high-fat diet was continually given to the treated obese mice for a subsequent two months before the evaluation of the anti-obesity properties of the commercial anti-obesity drug (Orlistat) and fermented jackfruit beverages was performed. The body weight and blood glucose levels were monitored during the treatment. Upon completion of the treatment, all mice were euthanized in a CO<sub>2</sub> chamber and blood samples were collected for the hematology analysis using an automated hematology analyzer (Exigo Blood Hematology Analyzer, Sweden). Some of the blood samples were stored in tubes containing anticoagulant EDTA-2k. Serum samples were obtained by centrifuging the blood samples (Centrifuge S417R, Eppendorf, CA, USA) at 4,000 rpm for 10 m under a chilled condition (4°C) and were measured using a clinical chemistry autoanalyzer (DIRUI CS300, China) for examining the liver function (i.e., alanine aminotransferase, ALT; alkaline phosphatase, ALP; aspartate aminotransferase, AST; total protein, TP) and lipid profiles (i.e., triglyceride, TG; high density lipoprotein, HDL; low density lipoprotein, LDL; cholesterol) of all the treated HFD-fed obese mice and normal

diet mice. The internal organs comprising the liver, kidney, spleen, and stomach were collected for histopathology analysis. The organs were fixed in neutral buffered 10% of formalin solution. All fixed organs were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined under a microscope (Leica, Germany). The animal experiment was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Malaysian Agricultural Research and Development Institute (MARDI). The protocol of the animal experiment was approved by the Animal Ethics Committee of MARDI (20170815/R/MAEC24) and the procedures were carried out in accordance with the approved guidelines. All animals were euthanized using CO<sub>2</sub> gas in a special chamber, and all efforts were made to minimize any suffering.

### Quantitative real-time polymerase chain reaction analysis

The total RNAs were extracted from the sample of the adipose tissue of the treated and control mice using an RNeasy Lipid Tissue Mini kit (Qiagen, Germany). The synthesis of the cDNAs was carried out using a QuantiNova Reverse Transcriptase kit (Qiagen, Germany) and predesigned PrimeTime Assay Std Probes 5' FAM/ZEN/3' IBFQ, which involved three reference gene probes: Actin-b (Actb) (Mm.PT.39a.22214843.g), (GADPH) (Mm.PT.39a.1), and hypoxanthine phosphoribosyl transferase-encoding gene (Hprt) (Mm.PT.39a.22214828). Additionally, nine target gene probes: glucose transporter 4 (Slc2a4) (Mm.PT.58.9683859), sterol regulatory element binding protein-1 (SREBP-1) (Mm.PT.58.8508227), adiponectin receptor 1 (Adipor1) (Mm.PT.58.42620207), nitric oxide synthase 2 (NOS2) (Mm.PT.58.5680554), tumor necrosis factor alpha (TNF- $\alpha$ ), transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) (Mm.PT.58.11254750), matrix metalloproteinase-2 (MMP2) (Mm.PT.58.9606100), interleukin 6 (IL6) (Mm.PT.58.12506617), and C-C motif chemokine ligand 2 (CCL2) (Mm.PT.58.42151692) were acquired from Integrated DNA Technology (IDT, Singapore). The quantitative polymerase chain reaction (qPCR) assays were conducted in a StepOnePlus real-time PCR system (Applied Biosystems, USA) with the PCR conditions for the sample analysis set according to the following: 95°C/3 m for the DNA polymerase activation (one cycle), 95°C/5 s for denaturation (40 cycles), and a final step of 60°C for 30 s for annealing and the extension. The gene expression was normalized to the expression of the reference genes of actin-b, GADPH, and Hprt, and relative gene expression analysis was performed using a 2<sup>-[delta-delta]</sup>Ct method.

### Gut microbiota analysis via 16S rRNA gene sequencing

The gut microbiota of the mice's fecal samples from the five groups were evaluated using 16S rRNA metagenomic sequencing. The mice's fecal sample was collected from each group, and bacterial genomic DNA was extracted using a NucleoSpin tissue mini kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. The 16S rRNA variable V3-V4 regions were amplified by the PCR using gene specific primers (16S V4:341F/806R) (341F: CCTAYGGGRBGCASCAG and 806R: GGACTAC-NNGGGTATCTAAT) and Phusion High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA). Sequencing libraries were constructed using the PCR products from each group and the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) according to the manufacturer's instructions. The sequencing of the 16S rRNA libraries was performed using an Illumina HiSeq 2500 platform with 250 bp paired end reads generated. The bioinformatics analysis on the sequencing reads were then conducted to obtain

clean operational taxonomic units (OTUs) prior to the annotation based on the GreenGene Database,<sup>14</sup> followed by a taxonomic assignment using an RDP classifier.<sup>15</sup> The shared and unique mice gut microbial community was presented in a Venn diagram, which was constructed using a Draw Venn Diagram online tool (<http://bioinformatics.psb.ugent.be/webtools/Venn/>).

### Analysis of short chain fatty acids (SCFAs)

Short chain fatty acids were extracted from the mice's fecal samples and quantified using gas chromatography according to the method described by Sew *et al*.<sup>16</sup> Approximately 0.3 g of the feces was added into a tube containing lysing matrix type E (MP Biomedicals, USA) and homogenized in 3 mL of sterile distilled water by vortexing to get the fecal suspension. The suspension was adjusted to a final pH 2 by adding 50  $\mu$ L of 10 M HCl and vortex vigorously for 10 m. The homogenate was then centrifuged at 10,000 rpm for 10 m at 4°C. The SCFAs containing the supernatant were filtered using a 0.22  $\mu$ m syringe filter and the fecal extracts were stored at -20°C until further analysis. The composition of the SCFAs was analysed using gas chromatography (Agilent 6890N, USA) equipped with a flame ionization detector. One  $\mu$ L of fecal extract was injected into the gas chromatography machine using an auto injector system. The major SCFAs (i.e., acetic acid, propionic acid, and n-butyric acid) were identified and quantified based on a standard calibration curve. All the samples were analysed in triplicate. The concentration of the SCFAs was recorded as the mean  $\mu$ mole per gram of feces ( $\mu$ mol/g). The ratio of each SCFA was determined from the data of the SCFAs before and after the treatment for each group.

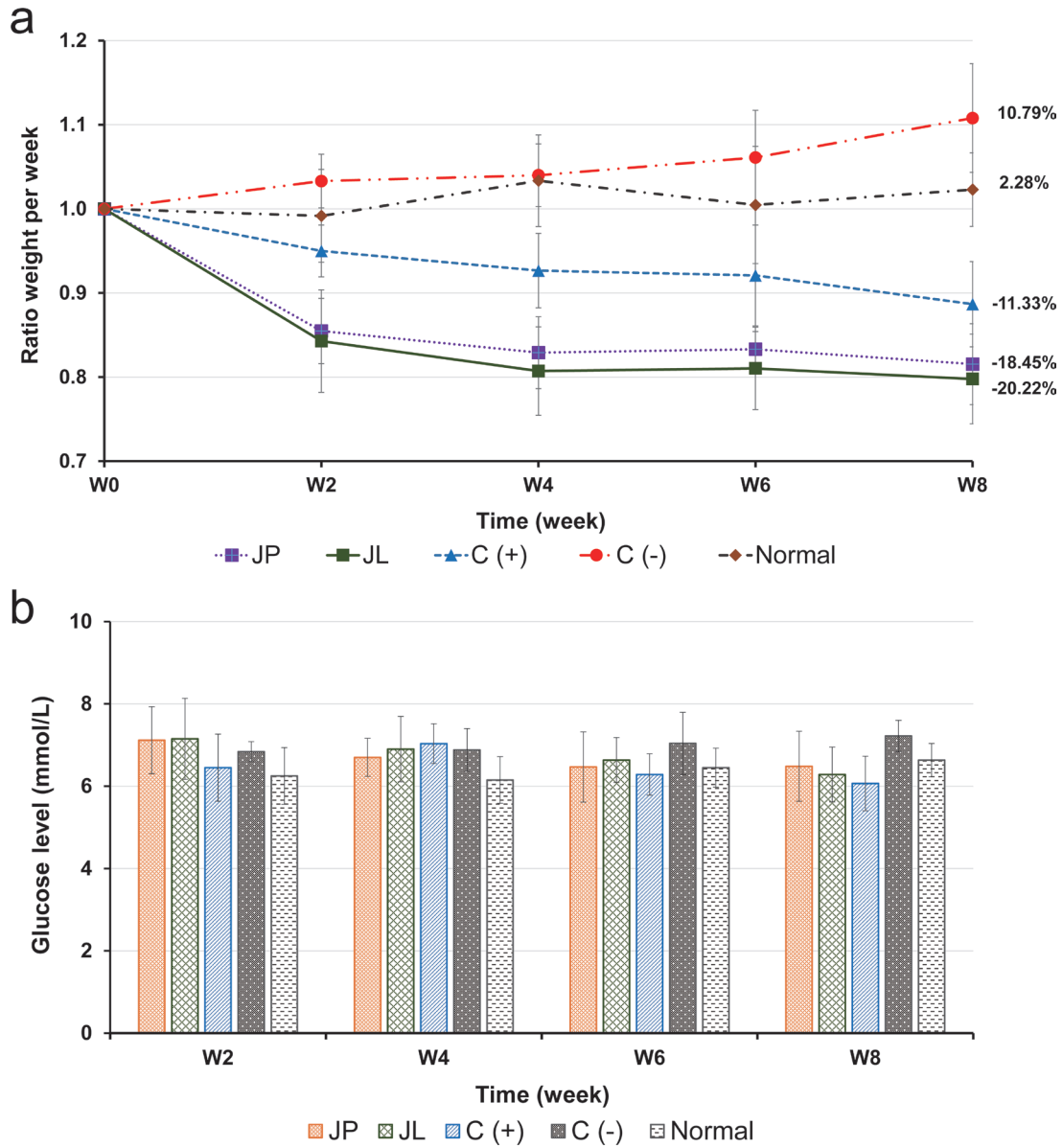
### Statistical analysis

All data are presented as the mean $\pm$ standard deviation of the replicated samples and subjected to one-way analysis of variance using statistical software, IBM SPSS Statistic 22.0 (IBM Corp., USA). The comparisons were performed by Duncan's test with statistical significance set at the level of  $p < 0.05$ .

## Results

### Anti-obesity study on HFD-fed obese mice

The present study evaluated the potential of SCOBY jackfruit beverages to counteract obesity using an HFD-fed obese mice model. Upon two months of SCOBY jackfruit beverages treatment, it was observed that the treated obese mice showed drastic reductions in body weight (18.45% in fermented jackfruit pulp, JP; 22.2% in fermented jackfruit leaves, JL) with higher reduction percentages than the obese mice group treated with Orlistat (11.3%) (Fig. 1a). In general, the blood glucose profiles of all the obese and normal diet mice were within the healthy levels (6–8 mmol/L) (Fig. 1b). In order to investigate the safety aspect of prolonged consumption of SCOBY jackfruit beverages and the commercial drug, blood serum biochemistry was also conducted. Clearly, there were increments in the levels of ALT, HDL, LDL, and cholesterol, where there were significant differences observed between the normal diet and HFD fed mice ( $p < 0.05$ ) (Table 1). However, the blood hematology profile of the mice treated with SCOBY jackfruit beverages and Orlistat revealed acceptable levels of white blood cells, lymphocytes, hemoglobin, hematocrits, red blood cells, and plate-



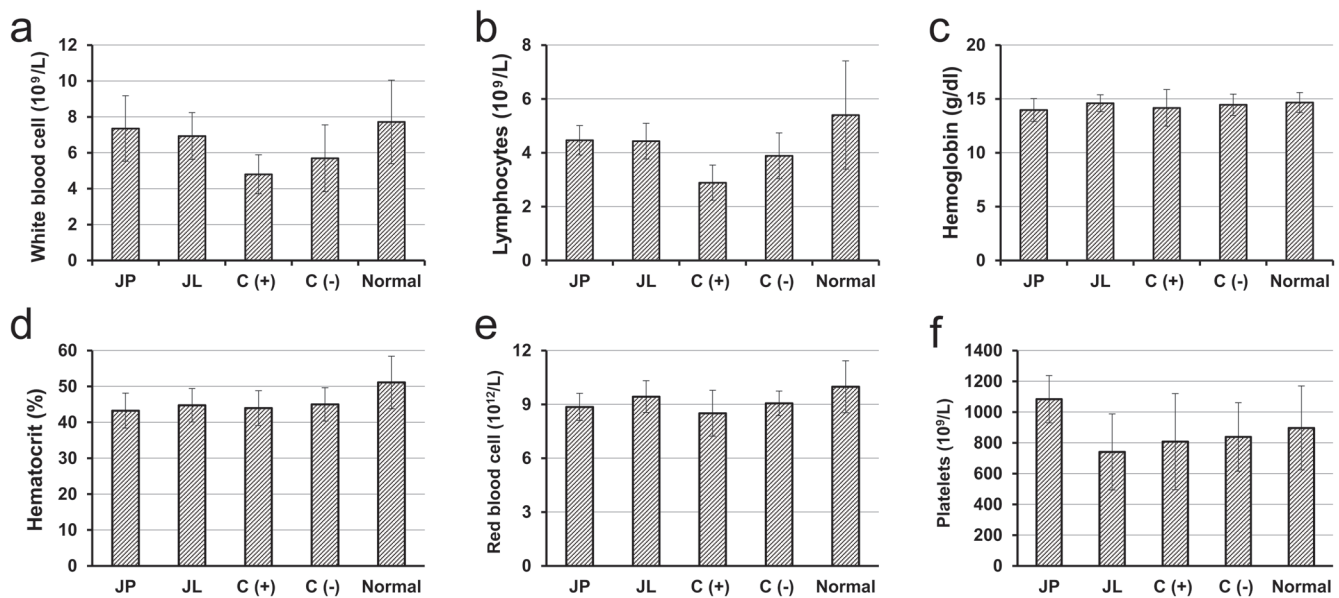
**Fig. 1. Antiobesity study of high-fat diet mice fed with various treatments for a duration of two months.** (a) ratio weight per week; (b) glucose level. High-fat diet, HFD, C(-); HFD-Orlistat, C(+); HFD-fermented jackfruit pulp, JP; HFD-fermented jackfruit leaves, JL; Normal diet mice, Normal.

**Table 1. Blood serum biochemistry profile of high-fat diet-fed mice fed with various treatments and normal diet mice**

Analysis	Liver function analysis				Lipid profile analysis			
	ALT (U/L)	AST (U/L)	ALP (U/L)	TP (g/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	CHOL (mmol/L)
Normal	48.40±5.10 <sup>c</sup>	134.57±29.82 <sup>a</sup>	76.43±12.84 <sup>a</sup>	71.62±7.10 <sup>a</sup>	1.09±0.20 <sup>a</sup>	1.21±0.23 <sup>b</sup>	1.31±0.30 <sup>b</sup>	2.37±0.42 <sup>c</sup>
C(-)	49.33±7.15 <sup>c</sup>	126.55±25.30 <sup>a</sup>	71.57±15.00 <sup>a</sup>	71.52±8.47 <sup>a</sup>	1.07±0.11 <sup>a</sup>	1.99±0.38 <sup>a</sup>	2.91±0.71 <sup>a</sup>	3.68±0.63 <sup>b</sup>
C(+)	67.25±6.65 <sup>a</sup>	127.83±34.37 <sup>a</sup>	72.30±19.12 <sup>a</sup>	77.12±6.30 <sup>a</sup>	1.24±0.31 <sup>a</sup>	2.12±0.83 <sup>a</sup>	2.57±0.34 <sup>a</sup>	4.07±0.47 <sup>b</sup>
JL	59.40±6.50 <sup>ab</sup>	151.20±20.09 <sup>a</sup>	77.50±12.57 <sup>a</sup>	80.90±6.60 <sup>a</sup>	0.72±0.09 <sup>b</sup>	2.27±0.52 <sup>a</sup>	3.38±1.35 <sup>a</sup>	4.91±0.59 <sup>a</sup>
JP	51.25±11.41 <sup>bc</sup>	158.80±26.31 <sup>a</sup>	82.00±12.46 <sup>a</sup>	80.48±5.69 <sup>a</sup>	1.15±0.44 <sup>a</sup>	2.49±0.60 <sup>a</sup>	3.35±1.06 <sup>a</sup>	5.26±0.46 <sup>a</sup>

<sup>abc</sup>Different letters in the same column show the significant differences ( $p < 0.05$ ). ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; TP, total protein; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; CHOL, cholesterol; C(-), high-fat diet (HFD) without treatment; C(+), HFD-Orlistat; JP, HFD-fermented jackfruit pulp; JL, HFD-fermented jackfruit leaves; Normal, Normal diet mice.





**Fig. 2. Hematology profile of high-fat diet mice fed with various treatments for a duration of two months.** (a) white blood cell; (b) lymphocytes; (c) hemoglobin; (d) hematocrit; (e) red blood cell; (f) platelets. High-fat diet, HFD, C(-); HFD-Orlistat, C(+); HFD-fermented jackfruit pulp, JP; HFD-fermented jackfruit leaves, JL; Normal diet mice, Normal.

lets, thus indicating they were safe for consumption (Fig. 2).

#### Quantification real-time polymerase chain reaction

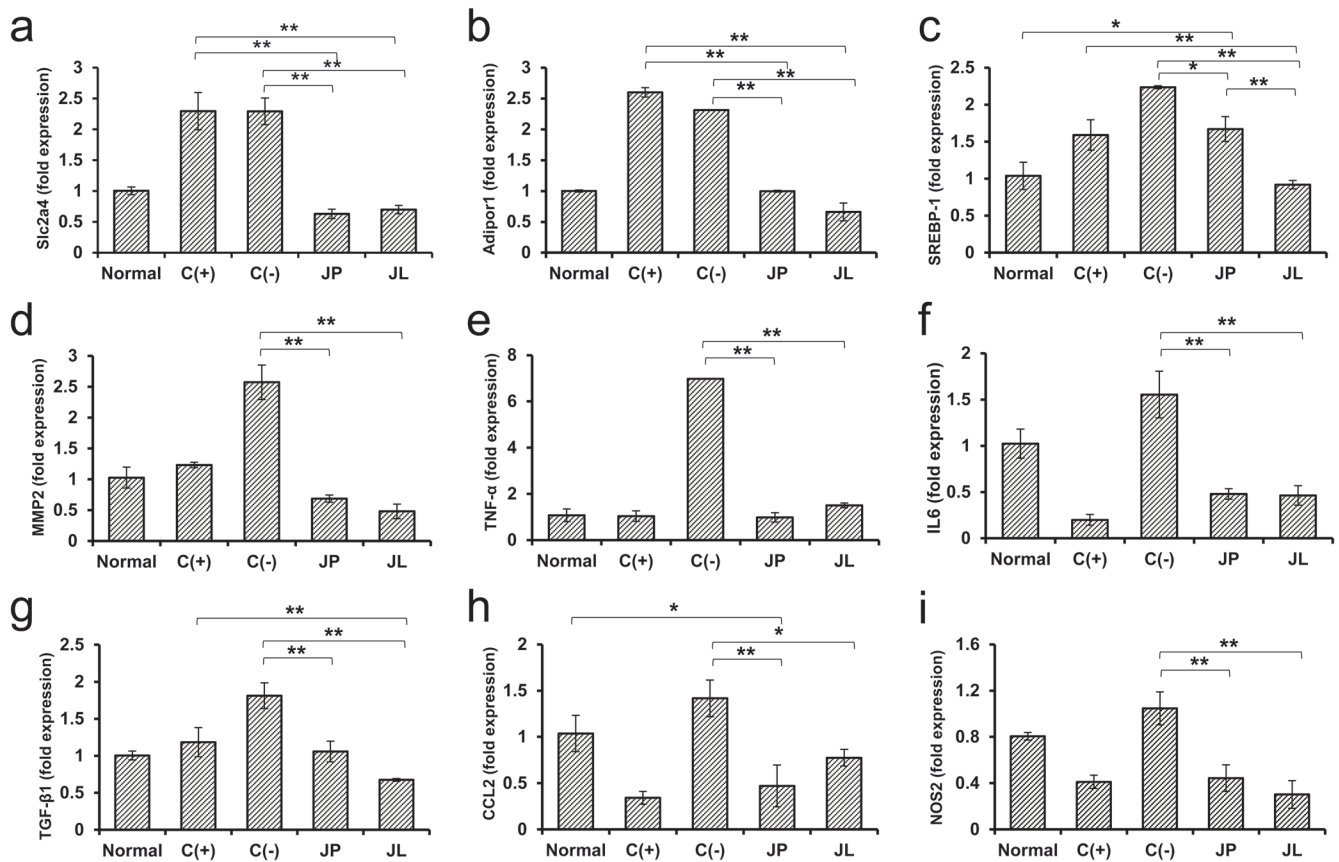
Obesity is known to be a chronic metabolic disease with low-grade inflammation. The molecular effects of the SCOBY jackfruit beverages on obese-induced inflammation were investigated *via* a gene expression analysis of the adipocyte genes involved in the glucose metabolism (Slc2a4), lipid metabolism (Adipor1, SREBP-1, and MMP2), and inflammation (TNF- $\alpha$ , NOS2, TGF- $\beta$ 1, IL6 and CCL2) (Fig. 3). The Slc2a4 functions as an insulin-regulated facilitative glucose transporter significantly reduced their expressions upon the JP and JL diet interventions when compared to both the Orlistat treated and HFD untreated groups (Fig. 3a;  $p < 0.01$ ). Adiponectin receptor 1 (Adipor1) showed a similar gene expression profile with Slc2a4 with a significant decreased expression level following individual JP and JL treatment (Fig. 3b;  $p < 0.01$ ). The transcription factor that was involved in the adipogenesis and controlling the cholesterol and fatty acid biosynthesis; namely, SREBP-1, was found to downregulate its expression levels in both the fermented diet-treated groups (Fig. 3c;  $p < 0.01$ ). The gene that was involved in the fat mass accumulation MMP2 showed a 2.5-fold upregulation in the HFD untreated group. This was relative to the normal control that was found to downregulate to levels lower than the normal and Orlistat treated control groups (Fig. 3d;  $p < 0.01$ ). Additionally, the JP or JL diet intervention was shown to result in a significant downregulation of pro-inflammatory cytokines TNF- $\alpha$  and IL6 expressions when compared to the HFD untreated control (Figs. 3 e-f;  $p < 0.01$ ). Similarly, there were significant reductions of the inflammatory chemokines TGF- $\beta$ 1 and CCL2 expression levels shown in both the JP and JL treated groups in comparison to the HFD untreated group (Figs. 3g-h;  $p < 0.01$ ). The higher levels of expression of NOS2 in the inflamed adipose tissues of the HFD untreated group were able to be restored to a normal level and below in the JP and JL treated groups, respec-

tively (Fig. 3i;  $p < 0.01$ ).

#### Gut microbiota and short chain fatty acids

The gas chromatographic technique was used to quantify the predominant SCFAs (acetic acid, propionic acid, and butyric acid) in each of the fecal samples collected from the treated and non-treated HFD-fed obese mice. In general, obese mice treated with SCOBY jackfruit beverages showed significant improvement in the production of SCFAs than other mice treatment groups (Fig. 4). Treatment using fermented jackfruit beverage in particular was found to increase the content of the SCFAs significantly ( $p < 0.05$ ). The collected fecal samples from all mice groups were subjected to fecal microbial composition analysis by sequencing of the 16S rRNA V3-V4 region amplicon libraries using the Illumina 250 bp paired-end sequencing platform. Briefly, the 16S rRNA metagenomics sequencing analysis revealed that the *Firmicutes* and *Bacteroidetes* were the two dominant phylum populations in the gut microbiota. All treated HFD-fed obese mice were observed to have a lower relative abundance of *Firmicutes* than normal and the HFD mice (Table 2). Relatively, it was noted that the obese mice treated with SCOBY jackfruit beverages showed a higher ratio of *Bacteroidetes*: *Firmicutes* (0.52–0.80) when compared to the high-fat diet-induced obese mice without treatment (0.45).

The changes in the gut microbiome in obese mice that underwent different diet interventions were investigated. The sequencing analysis demonstrated that the gut microbiome of the SCOBY jackfruit beverages treated HFD-fed obese mice showed that the abundance of beneficial gut microbes (*e.g.*, *Bifidobacterium*, *Faecalibaculum*, *Akkermansia*, *Lactobacillus*, and *Bacteroides*) increased as summarized in Figure 5a. However, the Venn diagram analysis revealed that the majority of the gut microbiota compositions were shared among the different mice groups (Fig. 5b). Evidence from the histopathology analysis indicated that the SCOBY jackfruit beverages did not cause any inflammation to the liver, kid-



**Fig. 3.** Gene expression of Slc2a4 (a), Adipor1 (b), SREBP-1 (c), MMP2 (d), TNF-α (e), IL6 (f), TGF-β1 (g), CCL2 (h), and NOS2 (i) in the controls (positive and negative) and fermented diet treated groups (JP and JL) relative to Normal. The results were presented as the mean ± SEM. Significant differences between the individual fermented treated group and control group were marked with \* ( $p < 0.05$ ) and \*\* ( $p < 0.01$ ). JL, jackfruit leaves; JP, jackfruit pulp.

ney, spleen, and stomach tissues in treated obese mice, thus indicating the safe consumption of SCOBY jackfruit beverages (Fig. 6).

## Discussion

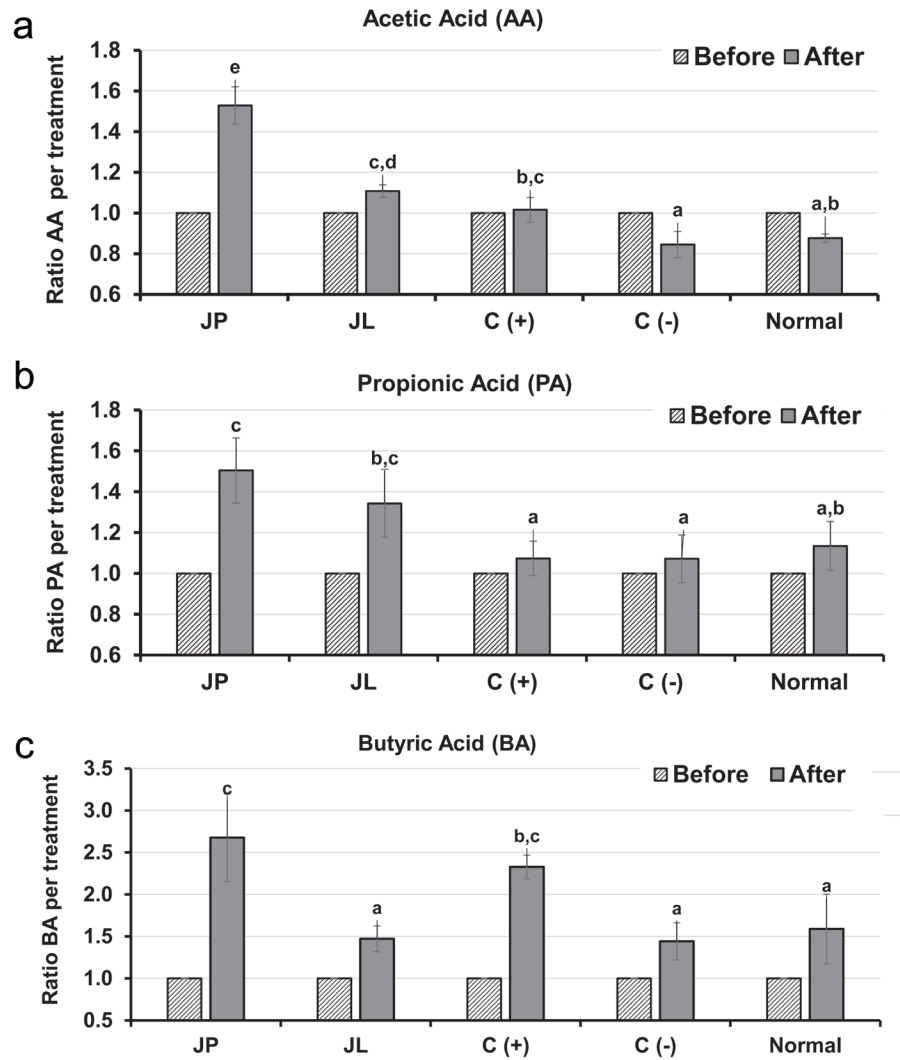
### Anti-obesity study on HFD-fed obese mice

In most cases, the obesity epidemics were caused by the combination of excessive food energy intake and sedentary lifestyle. The genetic factor, medical reasons, or psychiatric problems were also some of the limiting factors contributing to obesity.<sup>17</sup> As a result, the gut microbiota composition has emerged as one of the important factors influencing the pathophysiology of obesity. The role of gut microbial metabolites as functional signaling molecules has made them a potential nutritional and pharmacological target in the management of obesity.<sup>9</sup>

This present study evaluated the potential and effectiveness of two types of SCOBY jackfruit beverages as a new anti-obesity therapy to resolve the current obesity epidemic problem in a natural way *via* an HFD-fed obese mice model. A commercial anti-obesity drug, Orlistat, was used as a positive control for comparison purposes. Orlistat acts as a lipase inhibitor to reduce fat absorption; however, some people may experience diarrhea, abdominal pain, flatulence, and gastrointestinal side effects.<sup>18</sup> Within two months of

treatment, it was observed that the obese mice treated with the SCOBY jackfruit beverages demonstrated a higher efficacy in reducing their body weight (18.45% in fermented jackfruit pulp, JP; 22.2% in fermented jackfruit leaves, JL) than a commercial anti-obesity drug (Orlistat, 11.3%). The blood glucose levels of the treated HFD-fed obese mice were monitored to observe for any undesirable effects, such as hyperglycemia or hypoglycemia due to the consumption of the SCOBY jackfruit beverages. In general, no hypoglycemia or hyperglycemia effect was observed in all the obese and normal diet mice, hence indicating no side effects occurred on the mice's blood glucose profiles after two months of continual treatment with the SCOBY jackfruit beverages.

The blood serum biochemistry and hematology profiles were conducted to investigate the safety aspect of the prolonged consumption of the SCOBY jackfruit beverages and commercial drugs. The ALP, AST, TP, and TG content of most of the HFD-fed obese mice displayed no significant difference ( $p > 0.05$ ) from normal diet mice. Although the blood biochemistry profiles of the HFD-fed obese mice showed a minor increment in the readings, those HFD-treated mice were found physically active with no sickness symptoms observed. Furthermore, additional evidence of data from the blood hematology profiles revealed that the SCOBY jackfruit beverages and Orlistat were safe for consumption, as the levels of the white blood cells, lymphocytes, hemoglobin, hematocrits, red blood cells, and platelets fell within the acceptable ranges. The overall findings obtained from this study confirmed



**Fig. 4. Short chain fatty acids profile of high-fat diet mice fed with various treatments for a duration of two months.** (a) acetic acid; (b) propionic acid; (c) butyric acid. High-fat diet, HFD, C(-); HFD-Orlistat, C(+); HFD-fermented jackfruit pulp, JP; HFD-fermented jackfruit leaves, JL; Normal diet mice, Normal.

the effectiveness of the SCOBY jackfruit beverages on alleviating the adverse effects of high-fat diet induced obesity and their safe consumption with no toxicity symptoms shown.

**Quantification real-time polymerase chain reaction**

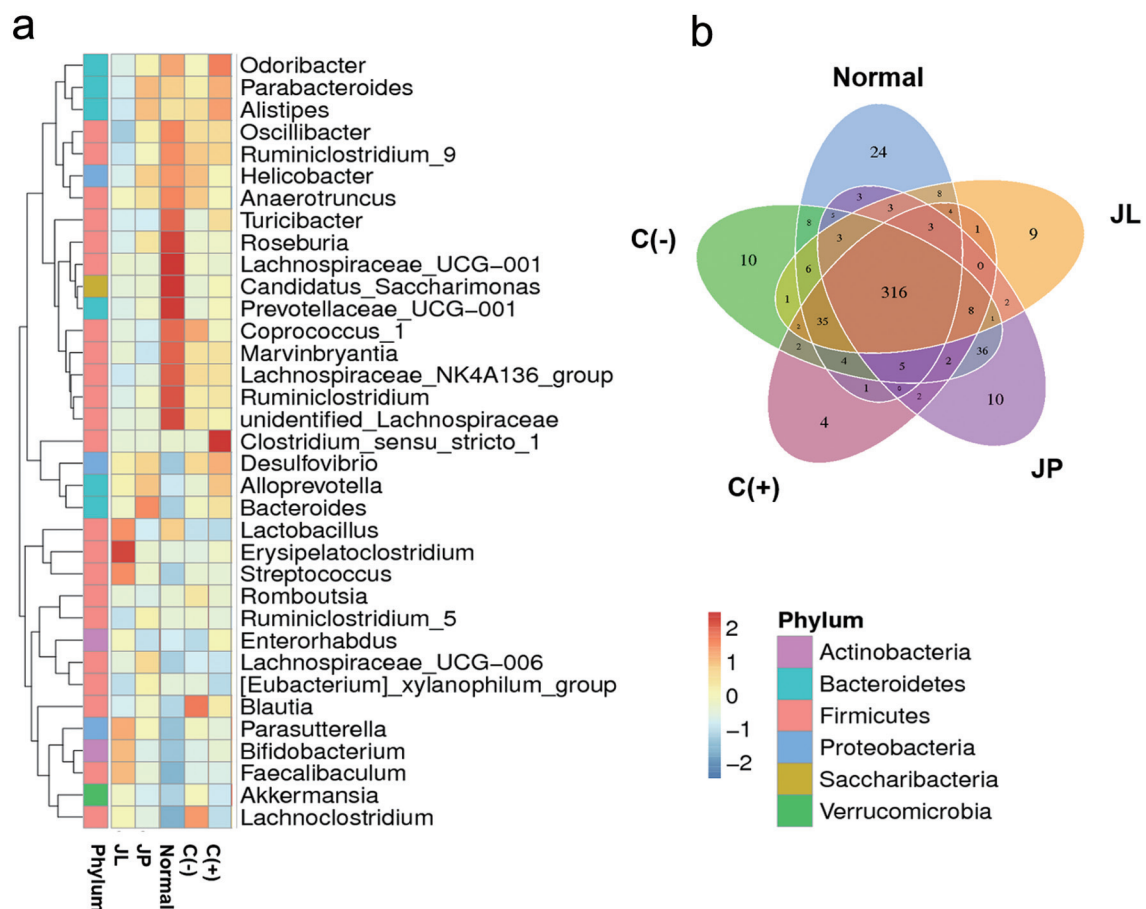
In order to assess the molecular changes due to the SCOBY jackfruit beverages dietary intervention, comparative gene expression analyses were performed using the total RNA extracted from the adipose tissues of the HFD-treated and untreated groups for measuring the expressions of the adipocyte genes involved in the cellular metabolism, such as glucose metabolism (Slc2a4), lipid metabolism (Adipor1, Srebf1, and MMP2), and inflammation (TNF- $\alpha$ , NOS2, TGF- $\beta$ 1, IL6, and CCL2). Our results showed that HFD-fed obesity resulted in the loss of the dynamic regulation of the expression of several metabolic genes in the adipose tissues. For instance, there was an increased expression level of Slc2a4, also known as GLUT4, which is a gene that is responsible for the insulin-stimulated glucose uptake in the adipose tissue of HFD-fed obese mice. The expres-

sion of Slc2a4 was significantly reduced upon the JP and JL diet interventions when compared to both the Orlistat treated and HFD untreated groups. These results coincided with an increase in the adiponectin receptor 1 (Adipor1) expression, consequently leading to an increase of the adenosin monofosfat protein kinase (AMPK) activity that enhanced the insulin-stimulated glucose and fatty acid uptake by the adipose tissues in obese mice.<sup>19</sup> The adiponectin receptor 1 (Adipor1) showed a similar gene expression profile with Slc2a4, with a significantly decreased expression level following the individual JP and JL treatments. The transcription factor that was involved in the adipogenesis and controlling the cholesterol and fatty acid biosynthesis (SREBP-1) was found to downregulate its expression levels in both the fermented diet-treated groups. Other studies also showed that continuous HFD could cause an elevation in the expression level of the transcription factor SREBP-1, which stimulated the gene activities related to the cholesterol and fatty acid biosynthesis in the adipose tissue.<sup>20</sup> The matrix metalloproteinase-2 (MMP2) was an essential proteinase actively involved during the formation of the obesity-mediated adipose tissue.<sup>21</sup> In this study, the gene involved in the fat mass accumulation MMP2 showed a 2.5-

**Table 2. Comparison of the relative abundance of the gut microbiota composition of high-fat diet-fed mice fed with various treatments with normal diet mice**

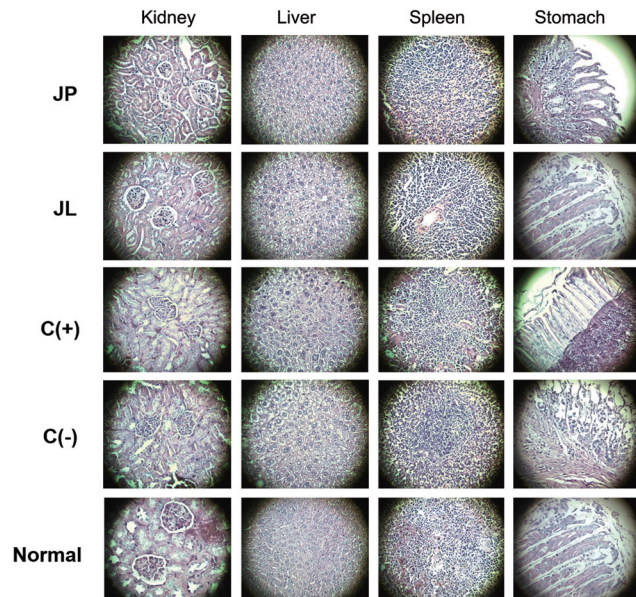
Taxonomy	Normal	C(-)	C(+)	JL	JP
Firmicutes	0.641614	0.605820	0.497338	0.551560	0.498693
Bacteroidetes	0.288663	0.273136	0.394176	0.288633	0.399290
Actinobacteria	0.007909	0.027185	0.038231	0.079790	0.028388
Verrucomicrobia	0.000557	0.030453	0.009057	0.025669	0.010421
Proteobacteria	0.044299	0.060197	0.054644	0.051933	0.059821
Saccharibacteria	0.011882	0.000891	0.002588	0.000625	0.001241
Spirochaetes	0.000021	0.000089	0.000317	0.000342	0.000671
Tenericutes	0.002943	0.000089	0.000042	0.000063	0.000072
Deferribacteres	0.000861	0.001474	0.002335	0.001220	0.000883
Cyanobacteria	0.001199	0.000118	0.001258	0.000139	0.000169
Fusobacteria	0.000004	0.000494	0	0.000008	0.000304
Others	0.000046	0.000055	0.000013	0.000017	0.000046
Ratio B: F	0.449901	0.450853	0.792572	0.523303	0.800673

B: Bacteroidetes; F: Firmicutes; C(-), high-fat diet (HFD) without treatment; C(+), HFD-Orlistat; JP, HFD-fermented jackfruit pulp; JL, HFD-fermented jackfruit leaves; Normal, Normal diet mice.



**Fig. 5. Metagenomic analysis on the gut microbiome of high-fat diet mice fed with various treatments for a duration of two months. (a) Heatmap profile; (b) Venn diagram. High-fat diet, HFD, C(-); HFD-Orlistat, C(+); HFD-fermented jackfruit pulp, JP; HFD-fermented jackfruit leaves, JL; Normal diet mice, Normal.**





**Fig. 6. Histopathology profile of various organ high-fat diet-fed and normal diet mice.** High fat diet, HFD, C(-); HFD-Orlistat, C(+); HFD-fermented jackfruit pulp, JP; HFD-fermented jackfruit leaves, JL; Normal diet mice, Normal.

fold upregulation in the HFD untreated group relative to the normal control. Inversely, the downregulated gene expression was observed in the SCOBY jackfruit beverages treated obese mice at the levels lower than normal and the Orlistat treated control.

The JP or JL diet intervention was shown to result in a significant downregulation of the pro-inflammatory cytokines TNF- $\alpha$  and IL6 expressions. A similar downregulated expression was also observed in the inflammatory chemokines TGF- $\beta$ 1 and CCL2 expression levels of both the JP and JL treated groups when compared to the HFD untreated control. The higher levels of NOS2 were detected in inflamed adipose tissues of the HFD untreated group in comparison to both the JP and JL treated groups after two months of treatment. Hence, all the above changes indicated a series of obesity development events which eventually triggered chronic inflammation in the adipocytes. This was clearly shown by the increased expression levels of the pro-inflammatory cytokines, such as TNF- $\alpha$  and IL6 as well as the inflammatory chemokines, such as TGF- $\beta$ 1 and CCL2. The expressions of these inflammatory cytokines and chemokines were linked positively with the degree of adiposity.<sup>22–25</sup> Thus, the inflammation process of the adipocytes had stimulated and up-regulated the NOS2 expression, which was a causal factor for the NOS2-derived nitric oxide (NO) species that promoted insulin resistance.<sup>26,27</sup> In contrast, following the JP and JL diet interventions, the above-mentioned gene expression levels were able to be restored to a normal level in both treated obese mice groups compared to the HFD untreated mice group ( $p < 0.05$ ). The molecular evidence here suggested that the SCOBY jackfruit beverages (JP and JL) were effective in preventing the development of obesity related pathogenesis under prolonged HFD consumption.

#### Gut Microbiota and short chain fatty acids

In fact, the long-term intake of HFD has been identified as one of the contributing factors to the increased risk of obesity, where it poses great influences on the gut intestinal microbiota by altering the com-

position and function of the microorganisms colonizing the gastrointestinal tract. The predominant SCFAs (acetic acid, propionic acid, and butyric acid) content in each of the fecal samples collected from treated and nontreated mice were determined using a gas chromatographic technique. In general, HFD-fed obese mice treated with SCOBY jackfruit beverages showed a significant improvement in the production of the SCFAs after two months of treatment. More significantly, treatment using fermented jackfruit pulp beverage was found to be more effective in increasing the content of the SCFAs. This finding was in agreement with a previous study where lactic acid bacteria inoculated fermented jackfruit pulp treatment was found to significantly increase the total content of the fecal SCFAs of immunosuppressed mice.<sup>28</sup> The SCFAs were produced by gut microbes as energy metabolites to increase the metabolic activity and proliferation of colonocytes, and play a defensive role in inflammation and atherosclerosis.<sup>29</sup> The SCOBY jackfruit beverages treated mice showed changes in the gut microbiota composition and increased the capability to generate obesity suppressing SCFAs, particularly acetic acid and propionic acid that contributed to a significant reduction of the body weight in previous HFD-fed obese mice studies.<sup>6,7</sup> Recent findings have confirmed that the role of propionic acid in regulating appetite and gut hormones *via* a free fatty acids receptor 3-independent mechanism would assist in the reduction of body weight.<sup>8,30</sup> The presence of multiple organic acids, particularly the acetic acid in SCOBY jackfruit beverages, would be the main metabolite with an anti-obesity effect by reducing the lipid deposition and increasing food satiety as reported in earlier studies. It was also reported that SCFAs propionate played a key role in appetite regulation and increased the secretion of peptide YY and glucagon like peptide-1, thus resulting in the reduction of weight gain in overweight adults.<sup>8</sup>

The collected fecal samples from all mice groups were subjected to a fecal microbial composition analysis using a 16S rRNA metagenomics sequencing technique. All treated HFD-fed obese mice were observed to have a lower relative abundance of *Firmicutes* than normal and HFD-fed obese mice. Moreover, the composition of intestinal microflora was influenced by the individual genotype and other environmental factors, such as diet, which played an important role. The microflora was dominated by four main phyla of bacteria: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* that maintained the homeostasis in the gut system, which concurred with the findings from the present study. A diverse composition of gut microbiota was found across different diet interventions with *Firmicutes* and *Bacteroidetes* as the two dominant phylum populations detected in the gut microbiota. Two months feeding on SCOBY jackfruit beverages increased the relative abundance of *Bacteroidetes* with a concurrent reduction of *Firmicutes*, consequently leading to an increased ratio of *Bacteroidetes*: *Firmicutes* in obese mice. The findings obtained in the present study were similar to those of Beh *et al.*,<sup>7</sup> where there was an anti-obesity effect of vinegar in HFD-induced obese mice.

The analysis of the gut microbiome and heatmap profile of the SCOBY jackfruit beverages treated obese mice revealed an increment in the beneficial abundance of the gut microbe (*e.g.*, *Bifidobacterium*, *Faecalibaculum*, *Akkermansia*, *Lactobacillus*, and *Bacteroides*) in comparison to the untreated HFD-fed obese mice. *Lactobacillus* and *Bifidobacterium* are common probiotic genera, which provide various health benefiting effects in the gut system *via* several mechanisms of action.<sup>31</sup> Similarly, the study conducted by An *et al.*<sup>32</sup> showed that *Bifidobacterium spp.* exerted anti-obesity and lipid-lowering effects in HFD-fed obese rats, which could explain the weight control potential by JL that contained a high abundance of these particular bacteria. On the other hand, *Faecalibaculum* was reported to produce lactic acid as a major metabolic end product,<sup>33</sup> and this lactic acid-producing bacteria was thought to exert anti-obesity effects.<sup>34</sup> Furthermore, it should

be mentioned that a relatively higher abundance of *Akkermansia spp.* was observed upon the JL treatment, where anti-obesity characteristics, such as regulating glucose metabolism, intestinal immunity and strengthening the intestinal barrier have recently been associated with these bacteria.<sup>35</sup> These results were consistent with the findings from previous studies where successful weight reduction in an obese human was mainly due to increased numbers of *Akkermansia* in their fecal samples.<sup>36</sup> In contrast, it was shown that the abundance of *Blautia spp.* was reduced markedly in both the jackfruit diet-treated groups when compared to the untreated control, and this genus was positively correlated with visceral fat accumulation.<sup>37,38</sup> Hence, the present data suggested that SCOBY jackfruit beverages helped in promoting healthier gut microbiota. Even though the Venn diagram displayed a majority of gut microbiota composition (at the OTUs basis) that was shared among the different mice groups, the unique microbiota composition shaped under the SCOBY beverage diet interventions significantly improved the health condition in the aspect of body weight control and reduction. Evidence from the histopathology analysis on the liver, kidney, spleen, and stomach tissues of the SCOBY jackfruit beverages treated obese mice showed no inflammation, thus indicating the safe consumption of SCOBY jackfruit beverages and their great potential as a food remedy in the management of weight control.

### Future directions

Fermented jackfruit beverages are not only appetizing, but also valuable for their proven health benefits. Microbial activity occurring during fermentation results in the increased bioavailability of micronutrients present in jackfruit pulp and leaves for the body to absorb and confer functional health benefits on the host. In relation to this, it would be expected that underlying complex synergistic mechanisms between functional compounds would be involved. Therefore, it would be of our great interest if the above-mentioned molecular mechanisms of the SCOBY jackfruit beverages would be elucidated in the future. Our forthcoming aim would also be to work closely with the private sector for larger scale production of fermented JP and JL to optimize the process parameter. In addition, further study would focus on the human obese populace for a more conclusive application of these SCOBY jackfruit beverages in weight reduction and management perspectives.

### Conclusions

This study provided multidimensional evidence that SCOBY jackfruit leaf and pulp beverages were effective in combating obesity and obesity related pathogenesis. Alterations in the expression of the genes involved in the adipocyte's metabolism gut microbiota, and SCFAs were found to be improved and recovered to a healthier state with significant weight loss in HFD-fed obese mice after being treated with SCOBY jackfruit beverages. The blood and histopathology profiles confirmed the safe consumption of SCOBY jackfruit beverages with no side effects observed. In consideration of the rapid increase of the obesity rate scenario, the present findings implied that SCOBY jackfruit beverages would potentially be used as a novel therapeutic strategy for weight management control in a natural way.

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### Conflict of interest

All authors declare that there was no conflict of interest.

### Author contributions

SPK developed technology for producing SCOBY jackfruit beverages; YSS and SS assisted in the qPCR gene expression and gut microbiome analysis; SAS and SM carried out the animal work and blood sample analysis; RA assisted in the preparation of the SCOBY jackfruit beverages; SPK and YSS contributed on the draft writing and other authors critically revised the manuscript. All authors made a significant contribution to this study and approved the final manuscript.

### Ethical Approval

The animal experiment was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Malaysian Agricultural Research and Development Institute (MARDI). The protocol was approved by the Animal Ethics Committee of MARDI (20170815/R/MAEC24), and the procedures were carried out in accordance with the approved guidelines. All animals were euthanized using CO<sub>2</sub> gas in a special chamber, and all efforts were made to minimize any suffering.

### Data Sharing Statement

The data used in supporting the findings of this study are included within the article.

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