

Combined effect of durian (*Durio zibethinus* Murr.) and β -glucan on glycaemic response and food intake in male rats

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Abstract

Durian (*Durio zibethinus* Murr.) is notorious for its stench. However, durian has a smooth texture and creamy taste, and is rich in macronutrients, bioactive compounds, and volatile compounds. These qualities could be useful in combination with other food components such as β -glucan. β -Glucan is a type of soluble fibre that has been approved by the European Food Safety Authority (EFSA) for beneficially reducing the postprandial glucose response. The aims of this study were to determine the total polyphenol content in durian and to determine the combined effects of durian and β -glucan on glycaemic response and food intake in rats. The total polyphenols content in the durians (variety D24) was 131.40 (22.20) mg GAE/100 g fresh weight. Durian significantly reduced ($p < 0.05$) postprandial glucose responses at 15, 30, 60, and 120 mins compared with control. A combination of durian and β -glucan significantly ($p < 0.05$) reduced glucose responses at 30, 60 and 90 mins compared with control. A combination of durian and β -glucan significantly ($p < 0.05$) reduced glucose responses at 120 mins compared with control and β -glucan. Food intake of the durian + β -glucan group was significantly ($p < 0.05$) lower than the control, durian and β -glucan groups. This study suggests that a combination of durian and β -glucan potentially reduces glycaemic response and food intake but this needs to be tested in humans.

1. Introduction

Durio zibethinus Murr., commonly known as durian, is widely dubbed the 'King of Fruits'. Recent research has shown that durian is rich in macronutrients (carbohydrate and sugar), bioactive compounds and dietary fibre (A Aziz and Mhd Jalil, 2019). Although durian is rich in bioactive compounds, its use for the development of functional food is still limited. Durian features in a few local Malaysian condiments and delicacies such as *serawa* and *lempuk* (Mat Amin *et al.*, 2007; Ho and Bhat, 2015) but their high salt or sugar content limits their health benefits. Durian has cholesterol-lowering properties when fed to hypercholesterolaemic rats (Leontowicz *et al.*, 2008). Leontowicz *et al.* (2011) demonstrated anti-atherosclerotic properties of durian in rats. A diet comprising 5% durian by weight reduced total lipid and low-density lipoprotein cholesterol compared with a control diet (Leontowicz *et al.*, 2011).

Durian is rich in sugar and fat, and hence energy-dense (A Aziz and Mhd Jalil, 2019). The total sugar

concentration of different durian varieties ranges from 3 g to 20 g per 100 g fresh weight in Malaysian, Thai and Indonesian varieties (A Aziz and Mhd Jalil, 2019). Despite the high sugar content, durian supplementation reduced post-prandial glucose and insulin responses in healthy volunteers compared with watermelon, papaya and pineapple (Robert *et al.*, 2008). The same study showed that the glycaemic index of durian was significantly lower than that of other tropical fruits. This could be due to the presence of polyphenols, potassium or fat. Our previous review showed that durian is rich in polyphenols with 374 mg gallic acid equivalent, 601 g potassium, and 5 g fat per 100 g fresh weight (A Aziz and Mhd Jalil, 2019). The fat content is important: high energy density meals with a high fat load significantly reduced gastric emptying by 22% compared with low energy density, high-protein load meals (Luscombe-Marsh *et al.*, 2013). Delayed gastric emptying is associated with a lower glycaemic response after a meal and vice versa (Marathe *et al.*, 2019). The rate of gastric emptying depends on factors such as the food matrix (liquid vs solid) and macronutrient composition (fat,

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protein and carbohydrate) (Phillips *et al.*, 2015). The presence of dietary components such as soluble dietary fibres (pectin, guar gum, polydextrose and β -Glucan) reduced the gastric emptying rate compared with control (Salleh *et al.*, 2019).

β -Glucan occurs in cereal grains. It is contained in oat and barley endosperm cell walls (Delaney *et al.*, 2003; Theuwissen and Mensink, 2008). β -Glucan is a functional ingredient with numerous health benefits, e.g. increased perceived satiety, reduced food intake and reduced postprandial glucose response (Salleh *et al.*, 2019). Previous studies showed β -glucan beneficially reduced glucose response when prepared in bread and biscuits (Kerckhoffs *et al.*, 2003; Hartvigsen *et al.*, 2014). β -Glucan is not digestible in the stomach but is metabolised by intestinal bacteria into short-chain fatty acids (SCFA) such as acetate, propionate and butyrate (Nishimura *et al.*, 2017). SCFAs, especially propionate, have been implicated in appetite regulation and might reduce body weight through stimulation of gut hormones peptide YY (PYY) and glucagon-like peptide 1 (GLP-1) (Chambers *et al.*, 2015). Barley β -glucan increased gut hormones PYY and GLP-1 and subsequently reduced food intake and improved insulin sensitivity in mice (Miyamoto *et al.*, 2018). It has been suggested that the increased levels of gut hormones PYY and GLP-1 were due to the increased levels of acetate, propionate and butyrate (Miyamoto *et al.*, 2018). Huang *et al.* (2011) showed an increased level of cholecystokinin (CCK) after β -glucan supplementation in mice. These findings suggest that β -glucan could beneficially reduce postprandial blood glucose response and reduced food intake. β -Glucan could hence be used as an active ingredient for the development of functional food in conjunction with other food components such as durian. This study aimed to determine the effect of combining β -glucan and durian in a liquid formulation on food intake and blood glucose response.

2. Materials and methods

2.1 Total polyphenols content

2.1.1 Extraction

Fresh durian sample (D24 variety, 5 g) was weighed and mixed with 250 mL of 70:30 acetone: water (v/v). The mixture was mixed for 3 mins in a laboratory blender at medium speed. The mixture was then placed in a shaking water bath at 20°C for 60 mins. Finally, an aliquot (7.5 mL) was centrifuged, at 4°C, at 4000 rpm for 10 mins. The supernatant was used for total polyphenols analysis (Lutz *et al.*, 2015).

2.1.2 Determination of total polyphenols content

Durian extract (2.5 mL) was mixed with 1.25 mL of Folin-Ciocalteu reagent, 3.75 mL sodium carbonate and 17.5 mL distilled water. The mixture was incubated for 30 mins at 37°C. The absorbance was measured at 765 nm against reagent blank. Gallic acid in the range of 0–100 μ g/mL was used as an external standard. Total polyphenols content is expressed as mg gallic acid equivalents (GAE)/g fresh weight (Singleton *et al.*, 1999).

2.2 Preparation of formulations (durian and β -Glucan)

Durian (variety D24) was purchased from a local supplier (Chabang Tiga, Terengganu, Malaysia). The dose of durian formulations was calculated according to Robert *et al.* (2008). Fresh durian pulp (193.0 g) was needed to prepare standardized formulation containing 50 g of available carbohydrate. The formulations were converted to a rat dose using a human-equivalent dose (HED) (Nair and Jacob, 2016). HED was calculated as follow: $193.0 \text{ g durian pulp}/60 \text{ kg}^* = 3.23 \times 6.2 = 20.03 \text{ g/kg}$ (*based on 60 kg weight of human) (USFDA, 2005). The conversion coefficient of 6.2 was used to account for differences in body surface area between rats and human (Nair and Jacob, 2016).

The dose of the formulations for β -glucan was followed according to European Food Safety Authority (EFSA). EFSA recommends 4 g of β -glucan with 30 g of available carbohydrates to reduce glycaemic response (EFSA, 2011). After considering the product purity, 20 g of β -glucan (20% purity) (Biogrow Oat BG22, Selangor, Malaysia) was used to match the EFSA recommendation. The formulations were converted to a rat dose using a human-equivalent dose (HED) (Nair and Jacob, 2016). HED was calculated as follow: $20.0 \text{ g BG22}/60 \text{ kg}^* = 0.33 \times 6.2 = 2.05 \text{ g/kg}$ (*based on 60 kg human) (USFDA, 2005). Again, the conversion coefficient of 6.2 accounts for differences in body surface area between rats and human (Nair and Jacob, 2016).

2.3 Animal preparation

Ethical approval was obtained from UniSZA Animal and Plant Research Ethics Committee (UAPREC), Universiti Sultan Zainal Abidin (UAPREC/04/010). Thirty-two male Sprague-Dawley rats (120 - 150 g initial weight) were purchased from local research animal supplier (Chenur Supplier, Kajang, Selangor, Malaysia). The rats were acclimatized at room temperature (24–28°C) under a standard light/dark cycle and were allowed to normal diet and water.

The rats were randomly separated to four groups as follows: control ($n=6$, 2.32 g/kg Glucolin + 20 mL/kg

0.03% CMC); durian ($n=6$, 19.20 g/kg durian); β -Glucan ($n=7$, 2.00 g/kg β -Glucan) and durian + β -glucan ($n=8$, 19.20 g/kg durian + 2.00 g/kg β -Glucan). Table 1 shows the composition of the four formulations. Rats had unrestricted access to drinking water and were fed a normal chow diet (Altromin, Lage, Germany). Bodyweight was recorded at baseline and food intake was measured before (baseline) and for up to 24 hrs.

Table 1. Composition of different formulations used in the study

	Control	Durian	β -Glucan	Durian + β -Glucan
Glucolin (g)	2.6	-	-	2.5
Durian aril (flesh) (g)	-	19.8	19.8	-
BG22 powder (g)	-	-	4.3	4.3

All formulations contain similar total sugar and were prepared in 0.03% Carboxymethyl Cellulose (CMC) as the vehicle.

2.4 Postprandial glycaemic response

Postprandial glycaemic response was determined at 0 to 120 mins and incremental area under the curve (iAUC) calculated. Different formulations were administered after an overnight fast (12 hrs) by gastric intubation. Fasting (0 min) blood was obtained from tail snip and thereafter at 15, 30, 60, 90 and 120 mins after supplementation. Glucose levels were determined using a glucometer (Accu-Chek, Roche Diagnostics, Switzerland). Incremental area under the curve (iAUC) was measured using a calculator courtesy of Dr Thomas Wolever, University of Toronto, Canada.

2.5 Statistical analysis

Data were analysed using IBM SPSS Statistics for Windows (version 22.0) (IBM Corp., Armonk, New York, USA). According to the data distribution, continuous data are presented as the mean (standard deviation or standard error mean) or median (interquartile range). For multiple comparisons, one-way ANOVA was used to define mean

differences of glucose response between groups with Bonferroni posthoc test. Two-way repeated measure Table 2. Bodyweight and food intake of rats

Rats	Bodyweight (g)	24 hrs food intake (g/24 hrs)
Control ($n=6$)	335.70 \pm 46.64 ^a	29.07 \pm 0.59 ^a
Durian ($n=6$)	349.67 \pm 45.72 ^a	29.05 \pm 0.22 ^a
β -Glucan ($n=7$)	335.78 \pm 28.45 ^a	27.36 \pm 0.97 ^a
Durian + β -Glucan ($n=8$)	351.22 \pm 31.45 ^a	22.60 \pm 0.79 ^b

Values are expressed as mean \pm standard deviation for body weight and mean \pm standard error mean for 24 hrs food intake. Values with different superscripts are significantly ($p < 0.05$) different within the same column.

ANOVA was used to compare the differences in glucose response at different time points. Non-parametric Kruskal–Wallis test was used to compare the median of food intakes and incremental area under the curve among groups with Mann-Whitney test for multiple comparisons.

3. Results

3.1 Baseline body weight and changes in food intake

Table 2 shows baseline characteristics of the experimental rats. Bodyweight was in the range of 335.70 (46.64) to 351.22 (31.45) g. Median daily food intake was significantly lower in Durian + β -glucan compared to other groups (Table 2) ($p = 0.008$).

3.2 Postprandial glycaemic response

The total polyphenols content of durian (D24 variety) was 131.40 (22.20) mg GAE/100 g fresh weight. Table 3 and Figure 1 show the mean postprandial glycaemic response at different time points. The durian group showed a significantly lower postprandial glucose at 30, 60 and 90 mins with 8.35 mmol/L ($p=0.028$), 7.82 mmol/L ($p=0.014$) and 8.00 mmol/L ($p=0.042$) compared with control with 12.08 mmol/L, 10.28 mmol/L

Table 3. Glucose Response from 0 to 120 mins

Time (mins)	Blood glucose level (mmol/L) (mean \pm SEM)			
	Control (n = 6)	Durian (n = 6)	β -Glucan (n = 7)	Durian + β -Glucan (n = 8)
0	7.47 \pm 0.18 ^a	6.50 \pm 0.12 ^a	6.53 \pm 0.23 ^a	6.56 \pm 0.29 ^a
15	12.00 \pm 1.00 ^{a,*}	10.03 \pm 0.75 ^a	10.31 \pm 0.86 ^a	10.61 \pm 0.62 ^{a,*}
30	12.08 \pm 1.24 ^a	8.35 \pm 0.56 ^b	10.03 \pm 0.36 ^{a,b,*}	9.38 \pm 0.82 ^{a,b,*}
60	10.28 \pm 0.73 ^a	7.82 \pm 0.49 ^b	8.80 \pm 0.37 ^{a,b,*,**}	8.19 \pm 0.35 ^{b,**}
90	9.63 \pm 0.20 ^a	8.00 \pm 0.54 ^b	8.67 \pm 0.34 ^{a,b,*,**}	7.99 \pm 0.33 ^{b,**}
120	10.00 \pm 0.39 ^{a,*}	8.10 \pm 0.39 ^b	9.51 \pm 0.17 ^{a,b,*}	8.34 \pm 0.32 ^b

Values are expressed as mean \pm standard error mean.

Values with different superscripts are significantly ($p < 0.05$) different within the same row.

*Significant differences between fasting (0 mins) with other time points (within column).

**Significant difference between 15 mins with other time points (within column).

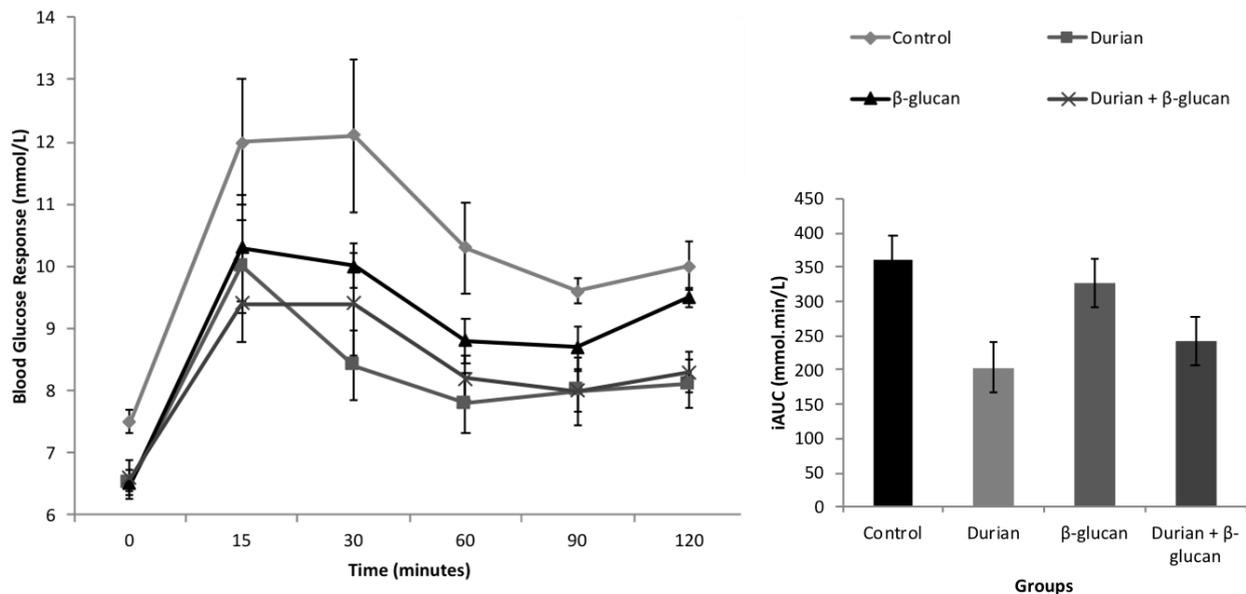


Figure 1. Mean glucose response and iAUC of all groups. Values are expressed as mean±SEM (n = 6-8). iAUC: incremental area under the curve

L and 9.63 mmol/L, respectively. The durian group showed a significantly lower glucose response at 120 mins with 8.10 mmol/L ($p=0.004$, 0.036) compared with the control and β-glucan with 10.00 mmol/L and 9.51 mmol/L, respectively. The durian + β-glucan group showed significantly lower glucose responses at 60, 90 and 120 mins with 8.19 mmol/L ($p=0.029$), 7.99 mmol/L ($p=0.024$) and 8.34 mmol/L ($p=0.008$), respectively compared with control (10.28 mmol/L, 9.63 mmol/L and 10.00 mmol/L, respectively). However, there was no statistical difference between groups ($p>0.05$) for iAUC.

There were significant differences between 0 (fasting) with 15 and 120 mins for control. There were no significant differences in blood glucose level at different time points in the durian group. There were significant differences between 0 (fasting) and other time points except at 15 mins for the β-glucan group and also between 15 mins with 60 and 90 mins. The durian + β-glucan group showed significant differences between 0 (fasting) at 15 and 30 mins and also between 15 mins and 60 mins, and between 15 mins and 90 mins.

4. Discussion

The total polyphenols content (TPC) of Malaysian durian variety D24 was 131.40 (22.20) mg GAE/100 g fresh weight. The TPC was higher than in other Malaysian varieties such as *Chaer Phoy* (67.12 mg GAE/100 g FW), Durian D11 (71.13 mg GAE/100 g FW), Durian *Yah Kang* (80.45 mg GAE/100 g FW) and Durian *Ang Jin* (97.78 mg GAE/100 g FW) and also higher than in four Thai varieties (26.44–112.17 mg GAE/100 g FW) (Ashraf et al., 2011; Charoenkiatkul et al., 2015). TPC content is especially high in the *Monthong* variety (360 mg GAE/100 g FW) (Ashraf et

al., 2011). TPC varies, however, with factors such as ripening and ripe durian showed higher total polyphenol content than merely mature durian (Arancibia-Avila et al., 2008). Other factors such as altitude also affect fruit quality and polyphenol content, possibly due to variations in ultraviolet light intensity, day length, and temperature (Jaakola and Hohtola, 2010).

The next aim was to determine the effects of durian, β-glucan and durian + β-glucan on food intake and blood glucose response in rats. Previous meta-analysis showed a significant reduction in the energy intake of individual β-glucan and there was a small to medium and non-significant effect size of β-glucan either in the liquid or solid food matrix on energy intake (Salleh et al., 2019). In our animal study, a combination of durian and β-glucan significantly ($p=0.008$) reduced 24 h food intake compared with other groups. The effects could be mediated by the released of satiety hormones. Satiety hormones could play a role in reduced hunger and positively reduced food intake (Lawley and Walker, 2005). Ghrelin is one of the gut hormones secreted from the stomach that stimulates food intake (Wren et al., 2001). Cholecystinin (CCK), peptide YY (PYY) and glucagon-like peptide (GLP-1) are released from the intestine and might inhibit food intake through the gut-brain axis (Walker et al., 2005). Gut hormones could be stimulated by substances such as short-chain fatty acids (SCFA) (Canfora et al., 2015). The effect of fibre on satiety depends on its physicochemical properties and physiological effects in the GI tract (van Kleef et al., 2011; Wanders et al., 2011).

Viscous fibres are likely to be more effective in promoting satiation. When β-glucan is consumed, it will form a highly viscous solution in the stomach and small

intestine, stimulates gastric distension, slows down the gastric emptying and affects the levels of gut hormones involved in appetite and satiety (Juvonen *et al.*, 2009; Kristensen and Jensen, 2011). Slowing the rate of food ingestion resulted in delayed activation of mechanoreceptors and chemoreceptors in the gastrointestinal tract, which could prolong satiety (Janssen *et al.*, 2011; Chambers, Morrison and Frost, 2015). High molecular weight barley β -glucan with increased viscosity has been reported to attenuate glycaemic response, gastric emptying and *in vitro* starch digestion (Thondre *et al.*, 2013). Carlson *et al.* (2017) showed that both Oatwell (containing 22% oat β -Glucan) and pure β -glucan significantly ($p < 0.05$) increased propionate concentrations after 12 hrs of fermentation with maximum concentration attained at 24 hrs, compared with control fibres (xylooligosaccharides, whole fibre mixtures and pure inulin) (Carlson *et al.*, 2017). Another study showed increased SCFA levels (2-methyl-propanoic, acetate, butyrate, and propionate) after 2 months of barley β -glucan treatment in healthy subjects (Angelis *et al.*, 2015). Another study supplemented inulin-propionate ester in a randomized, double-blind, placebo-controlled study in fifty healthy volunteers for 24 weeks. The supplementation increased postprandial PYY and GLP-1 levels and reduced overall energy intake (Chambers *et al.*, 2015).

A study by Miyamoto *et al.* (2018) showed barley β -glucan (0.6% to 2.0%) increased PYY and GLP-1 and subsequently suppressed appetite and improved insulin sensitivity in mice fed with a high-fat diet. The supplementation significantly ($p < 0.05$) increased acetate and propionate production in the colon compared with control. This could be due to a significant increase in the number of *Actinobacteria* in the colon (Miyamoto *et al.*, 2018). *Actinobacteria* is one of the saccharolytic bacteria in the gut that is able to ferment soluble β -glucan to produce SFCA (Chakraborti, 2015; Binda *et al.*, 2018).

Durian contains ethyl propionate, methyl propionate and propyl propionate (A Aziz and Mhd Jalil, 2019). Hence, a combination of durian and β -glucan might have additional effects on SCFA production and possibly also on blood glucose response. This study showed that a combination of durian and β -glucan significantly ($p < 0.05$) reduced glucose levels at 60, 90 and 120 mins compared with control. Rats supplemented with durian showed significantly ($p < 0.05$) reduced glucose levels at 30, 60 and 90 mins compared with control. Durian is rich in fat and sugars but showed significantly lower glycaemic index (GI) in humans compared with other tropical fruits, specifically watermelon, papaya and pineapple (Robert *et al.*, 2008). Durian's low GI values might be due to its high fibre and fat content. Fat and

sugar contents are in the range of 1.6 to 5.3 g per 100 g fresh weight and 8 to 20 g per 100 g fresh weight, respectively in different durian varieties (A Aziz and Mhd Jalil, 2019). Fat has no direct effect on blood glucose response but could delay gastric emptying and subsequently slow the rate of glucose absorption (Hu *et al.*, 2001). Another study showed that supplementation with the *Monthong* durian variety (5% freeze-dried) reduced plasma glucose in cholesterol-enriched diets compared with control (Leontowicz *et al.*, 2007). Durian is rich in potassium in the range of 70 to 600 mg per 100 g fresh weight, comparable to potassium-rich banana (A Aziz and Mhd Jalil, 2019). A meta-analysis and systematic review by Carter *et al.* (2010) suggested a significant association of a higher intake of potassium-rich green leafy vegetables with lower risk of diabetes (Carter *et al.*, 2010). A recent experimental study showed that potassium chloride (KCl) supplementation improved fasting glucose levels in African Americans with prediabetes (Chatterjee *et al.*, 2017). However, there were no significant effects on glucose and insulin levels. Another explanation may be the presence of polyphenols. A recent review showed total polyphenol content (TPC) in the range of 21 to 283 mg/100 g fresh weight (A Aziz and Mhd Jalil, 2019). In the present study, TPC of durian was 131 mg/100 g fresh weight. Kim *et al.* (2016) showed that polyphenol-rich beverages (coffee, tea and chocolate) and fruits could inhibit α -amylase and glucosidase, inhibit glucose absorption in the intestine and reduce hepatic glucose output (Kim *et al.*, 2016). Hence, in this study, we proposed that potassium and polyphenols in durian could play a role in controlling blood glucose. However, this needs further investigation in animal or human studies.

This study showed that a combination of β -glucan with durian rather than β -glucan per se was more effective in reducing blood glucose level. High viscosity β -glucan effectively decreased glucose response compared with the low viscosity (Panahi *et al.*, 2007; Juvonen *et al.*, 2009). Viscosity of β -glucan depends on its molecular weight and food sources (e.g. barley, oats, mushroom) (Panahi *et al.*, 2007; Cleary *et al.*, 2007). A review by Tosh (2013) showed that 76% of treatments from a variety of processed foods containing 4 g of oat or barley β -glucan significantly reduced glycaemic response. This was in line with the European Food Safety Authority's recommendation of 4 g of β -glucan (either oats or barley) per 30 g available carbohydrate to reduce blood glucose response without adversely increasing insulin response (EFSA, 2011). In this study, however, we failed to observe any significant changes in blood glucose response for the β -glucan group. The purity of β -glucan used was 20% which could partly explain the observed outcome. However, this study

suggests that durian reduced blood glucose response and combination of β -glucan with durian reduced food intake. Further in vivo study with higher purity of β -glucan with defatted durian should be investigated in more detail.

Conflicts of interest

The authors declare no conflict of interest. The sponsor had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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