Supplementary Materials

Chemical composite of indigenous whole grain scented joha rice varietal prevents the type 2 diabetes in rats through ameliorating the insulin sensitization by IRS-1/AKT/PI3K signalling cascade

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Total carbohydrate content analysis

To measure the total carbohydrate, 100 g of rice seeds was weighed and powdered. Total carbohydrate was estimated by following the protocol described by us (Choudhury et al., 2020). Glucose was used as standard and different concentration gradients (0, 0.2, 0.4, 0.6, 0.8, 1.0 mg/mL) were made from 1 mg/mL.Briefly, Rice samples were hydrolysed with 2.5 N HCL and neutralised with sodium carbonate. Finally, to both the standard and sample 4mLAnthrone reagent was added and boiled. After rapid cooling absorptionwas measured in a spectrophotometer (MULTISKAN G0) at 630 nm (Hedge & Hofreiter, 1962; Pons et al., 1981). Totalcarbohydrate content was calculated per 100 g of thesample.

Total starch content analysis

To measure the total starch content the sample preparation method is same as total carbohydrate content. Previously described protocol ([11]Choudhury et al., 2020) was followed as glucose was used as positive control in different concentration gradients as (0, 0.2, 0.4, 0.6, 0.8, 1.0 mg/mL) from a stock of 1 mg/mL in distilled water. The powdered rice sample was homogenised in 80% ethanol (EtOH) to remove sugars. In the final step of the procedure anthrone reagent was added in both the sample and standard followed by heating in a water bath and subsequent rapid cooling. Finally, absorbance was measured ina MULTISKAN G0 spectrophotometer at 630 nm (Hedge &Hofreiter, 1962; Thayumanavan & Sadasivam, 1984).

Total soluble protein analysis

To measure the total soluble protein, the method described by us (Choudhury et al., 2020) was followed. The rice seeds (100 g) were weighed, powdered and dissolved in PBS (pH 7.4). Bovine serum albumin (BSA) was taken as standard and prepared as 1 mg/mL in PBS (pH 7.4). In the test sample a dye binding solution (coomassie brilliant blue G 250) is added and mixed well to develop a colour for 5 min. The red dye turns blue when it binds protein. The absorbance is read in a MULTISKAN G0 spectrophotometer at 595 nm (Bradford, 1976).

Mineral content analysis

The essential minerals present in rice sample were detected with Atomic Absorption Spectrophotometer (AAS) (SHIMADZU AA 7000; SHIMADZU Corp., Japan) following the standardised method described by us (Choudhury et al., 2020). Briefly, rice seeds (2 g) is measured and powdered followed by acid digestion with concentrated nitric acid till the sample becomes colourless. After that the samples were then diluted to 100 mL with distilled water and the analysis was carried out (Choudhury et al., 2020).

TableS1: Record of the micronutrients content

SL No	Rice Sample	Mn	K	Ni	Pb	Cr	Zn	Ca	Cu	Mg	Fe	Na
1	Kola Joha (KJ)	0.34 ± 0.03	11.52 ± 0.01	0.09 ± 0.00	ND	0.12 ± 0.09	ND	39.11 ± 0.08	ND	3.41 ± 0.37	0.44 ± 0.05	3.44 ± 0.77
2	Ranjit (RR)	0.27 ± 0.02	4.93 ± 0.07	0.10 ± 0. 06	ND	ND	ND	26.16 ± 0.42	0.026 ± 0.01	2.47 ± 0.49	0.23 ± 0.09	4.59 ± 0.81

**ND= Not detected or not measurable.*

Sub-acute Toxicity Study

The sub-acute toxicity was examined in 5 male wistar rats of weight 80-100 g for a period of 21 days. The rats were orally fed on a single dose of methanolic (MeOH) extract of scented rice Kola Joha (KJ). The dose selected was 2000 mg/Kg body weight according to the OECD guidelines. The rice extract was dissolved in 0.3% CMC buffer of pH 4.45. The rats were then observed for 21 days for any kind of behavioural or toxicological changes.

Results: After a period of 21 days the rats showed no visible toxicological changes. The rats were examined for the following conditions.

Table S2: Observation sheet on21th day

Sl.no.	Observation	Single oral dose (2000 mg/kg)				
	RAT ID	I	II	III	IV	V
1	Lethality	No	No	No	No	No
2	Convulsions	No	No	No	No	No
3	Straub tail	No	No	No	No	No
4	Sedation	No	No	No	No	No
5	Excitation	No	No	No	No	No
6	Jumps	No	No	No	No	No
7	Loss of balance	No	No	No	No	No
8	Abnormal writhes	No	No	No	No	No
9	Piloerection	No	No	No	No	No
10	Stereotypies	No	No	No	No	No
11	Head twitches	No	No	No	No	No
12	Scratching	No	No	No	No	No
13	Abnormal respiration	No	No	No	No	No
14	Loss of righting reflex	No	No	No	No	No
15	Loss of corneal reflex	No	No	No	No	No
16	Defecation	No	No	No	No	No
17	Salivation	No	No	No	No	No
18	Lacrimation	No	No	No	No	No
19	Aggressiveness	No	No	No	No	No

Table S3: Animal food intake per week per group (in g) with details of survival record

Week	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI
Week 1	147.8 (n=6)	135 (n=6)	141.5 (n=6)	140.1 (n=6)	133.9 (n=6)	141 (n=6)
Week 2	146.1 (n=6)	133.3 (n=6)	140 (n=6)	134 (n=6)	139.3 (n=6)	143.4 (n=6)
Week 3	129 (n=6)	133 (n=6)	137.8 (n=6)	134.3 (n=6)	144 (n=6)	147.1 (n=6)
Week 4	122 (n=6)	134.6 (n=6)	132 (n=6)	134.6 (n=6)	149.4 (n=6)	142.9 (n=6)
Week 5	97.6 (n=6)	141 (n=6)	131.2 (n=6)	119.3 (n=6)	152 (n=6)	149 (n=6)
Week 6	124.5 (n=6)	167.8 (n=6)	139 (n=6)	158 (n=6)	155.6 (n=6)	153.8 (n=6)
Week 7	124.2 (n=6)	208.0 (n=6)	145 (n=6)	167.9 (n=6)	159 (n=6)	159.9 (n=6)
Week 8	167.9 (n=6)	200 (n=5)	148.8 (n=6)	138.4 (n=6)	162.2 (n=6)	165.1 (n=6)
Week 9	166.3 (n=6)	201.8 (n=5)	148 (n=6)	145 (n=6)	163.5 (n=6)	162.9 (n=6)
Week 10	168.3 (n=6)	202.2 (n=5)	154 (n=6)	149.8 (n=6)	159.7 (n=6)	164 (n=6)
Week 11	166.1 (n=6)	203.8 (n=5)	157 (n=6)	151.6 (n=6)	167 (n=6)	169 (n=6)
Week 12	200.6 (n=5)	214.8 (n=5)	159 (n=6)	154.1 (n=6)	172 (n=6)	173.6 (n=6)
Week 13	198.2 (n=5)	200 (n=5)	165 (n=5)	157.8 (n=6)	175 (n=6)	177 (n=6)
Week 14	197.8 (n=5)	186 (n=5)	169.9 (n=5)	161.5 (n=6)	179.5 (n=6)	175.8 (n=6)
Week 15	200.2 (n=5)	195.8 (n=5)	173.6 (n=5)	163.8 (n=6)	183.7 (n=6)	180.6 (n=6)
Week 16	197.4 (n=5)	195.6 (n=5)	177.1 (n=5)	168.3 (n=6)	182.7 (n=6)	182.3 (n=6)
Week 17	198.4 (n=5)	197 (n=5)	183 (n=5)	172.8 (n=6)	189.6 (n=6)	184 (n=6)
Week 18	199.6 (n=5)	198.6 (n=5)	186.6 (n=5)	186.5 (n=6)	194.2 (n=6)	183.8 (n=6)
Week 19	199.8 (n=5)	194.4 (n=5)	189 (n=5)	191.1 (n=6)	195.5 (n=6)	186 (n=6)

Week 20	199.4 (n=5)	196.2 (n=5)	196.7 (n=5)	193.5 (n=6)	196 (n=6)	186.6 (n=6)
Week 21	202 (n=5)	197.6 (n=5)	197 (n=5)	199.5 (n=6)	194 (n=6)	191.5 (n=6)
Week 22	203.8 (n=5)	199.4 (n=5)	201.3(n=5)	207.8 (n=6)	198.8 (n=6)	195.3 (n=6)
Week 23	204.6 (n=5)	197.8 (n=5)	200 (n=5)	210.6 (n=6)	203.8 (n=5)	199.6 (n=6)
Week 24	205.8 (n=5)	198.2 (n=5)	198.6 (n=5)	209.5 (n=6)	200.4 (n=5)	203 (n=6)

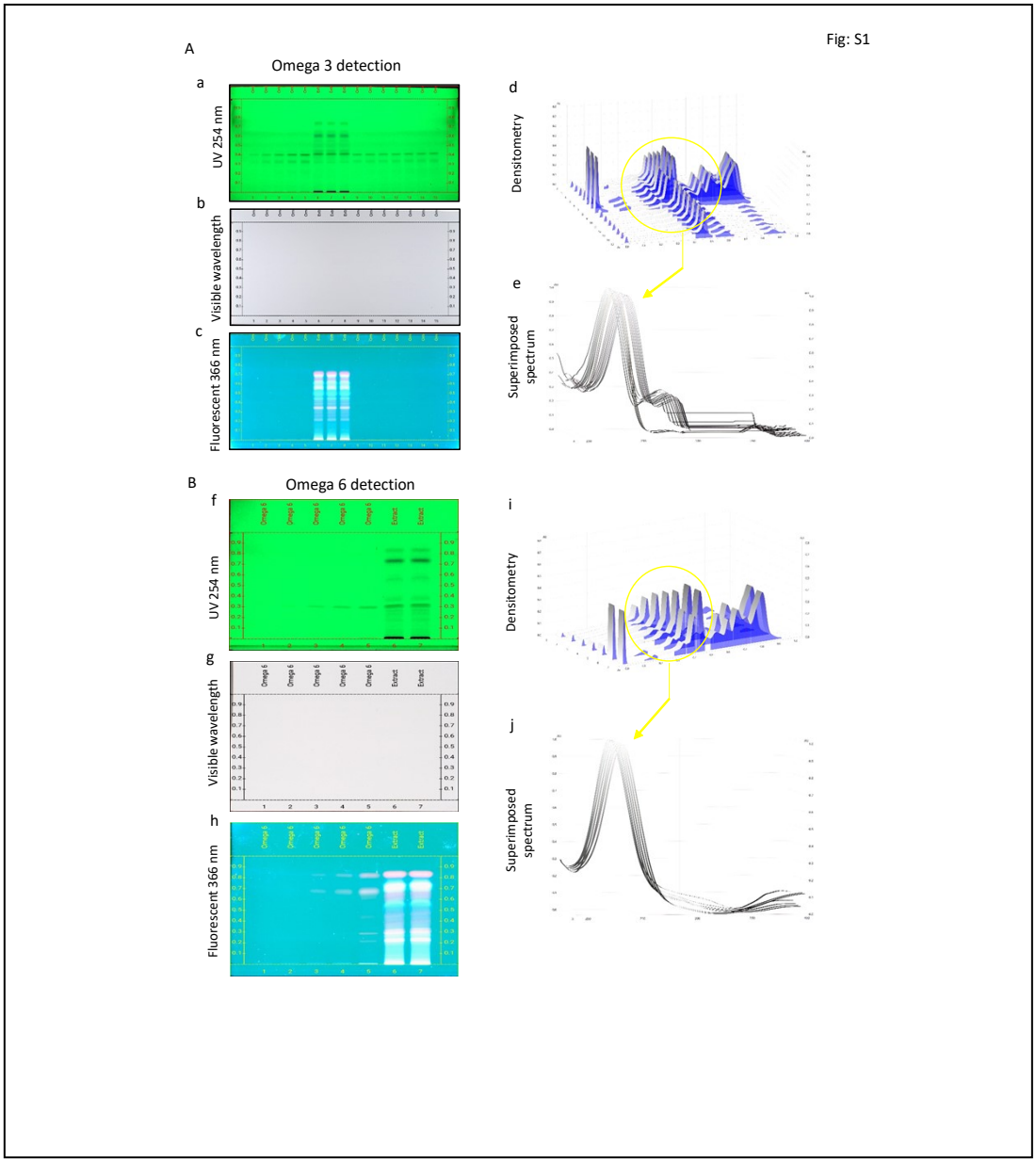

Fig. S1. (A). HPTLC fingerprinting of PCKJ for the detection of Linolenic acid (Omega-3 fatty acid) at different wavelengths (a) UV 254 nm wavelength, (b) visible wavelength, (c) Fluorescent 366 nm, (d) Densitometry, (e) superimposed spectrum. [B] HPTLC fingerprinting of PCKJ for the detection of Linoleic acid (Omega-6 fatty acid) at different wavelengths (f) At UV 254 nm wavelength, (g) visible wavelength, (h) Fluorescent 366 nm, (i) Densitometry, (j) superimposed spectrum.

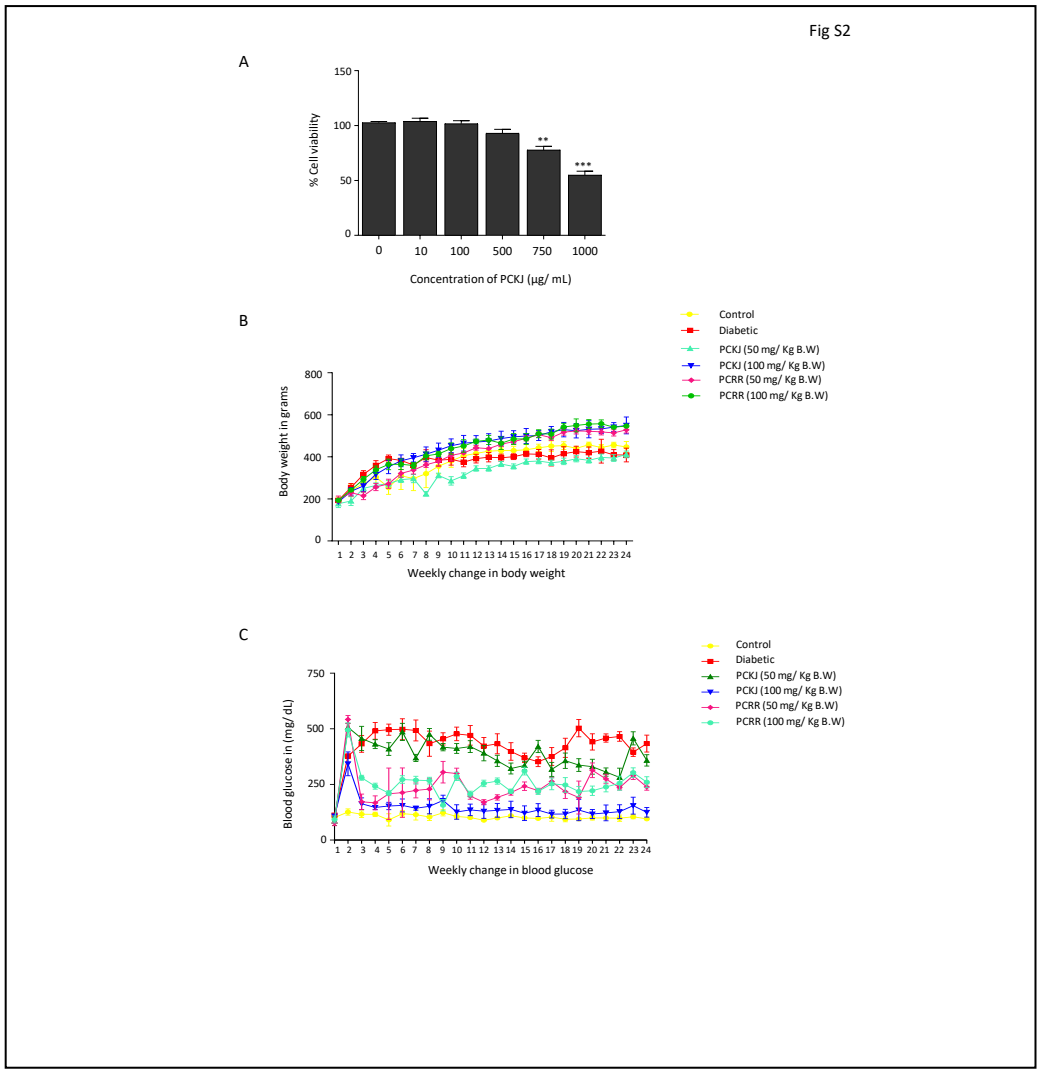

Fig. S2. (A) The effect of PCKJ on cell viability was analysed by trypan blue assay on L6 myotubes; % of viable cells after treatment with PCKJ dose (10, 100, 500, 750, 1000 (µg/mL)) for 24 h. Experiment was repeated thrice with reproducible results. Data was expressed as mean ± SD (n =3).*Statistically significant ($p<0.05$) compared to respective control and was calculated by one-way ANOVA followed by Dunnett's multiple comparison tests. (B) Body weight changes over a period of 24 weeks (in grams). (C). Blood glucose changes over a period of 24 weeks (in mg/ dL). All the data are represented as mean ± S.D with (n=6) animal in each group. *Statistically significant ($p < 0.05$) signifies the difference between control, HFHF (diabetic) and HFHF + treated (PCKJ 50 mg/ Kg B.W) HFHF + treated (PCKJ 100 mg/ Kg B.W) HFHF + treated (PCRR 50 mg/ Kg B.W) HFHF + treated (PCRR 100 mg/ Kg B.W) group. Data was calculated by one-way ANOVA followed by Tukey's multiple comparison tests in GraphPad Prism (9.3.0).

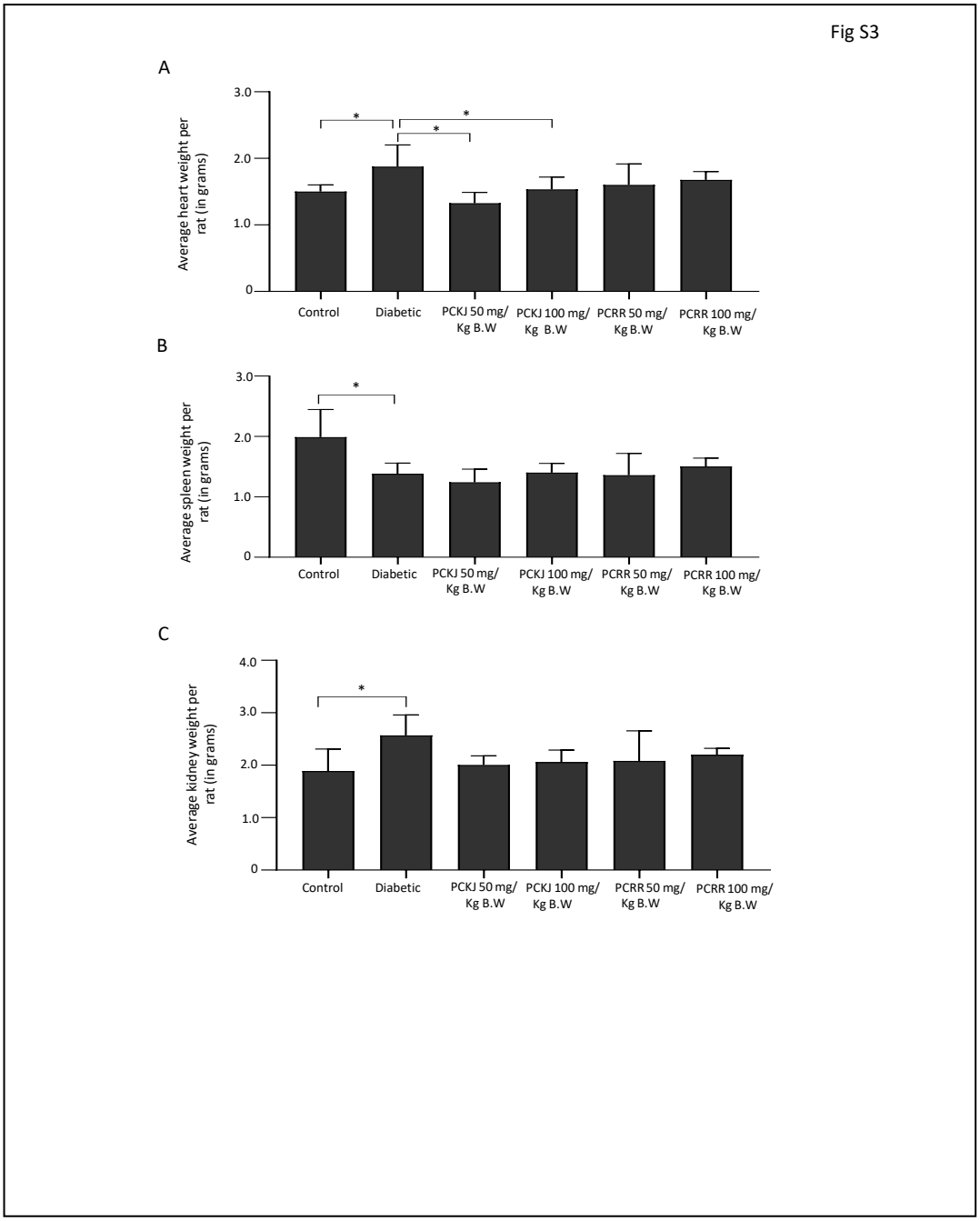

Fig. S3. The weight of various internal organs post-experiment animal sacrifice (in grams). (A) Weight of heart (in grams). (B) Weight of spleen (in grams). (C) Weight of kidney (in grams). Statistically significant ($p < 0.05$) signifies the difference between control, HFHF (diabetic) and HFHF + treated (PCKJ 50 mg/ Kg B.W) HFHF + treated (PCKJ 100 mg/ Kg B.W) HFHF + treated (PCRR 50 mg/ Kg B.W) HFHF + treated (PCRR 100 mg/ Kg B.W) group. Data was calculated by one-way ANOVA followed by Dunnett's multiple comparison tests in GraphPad Prism (9.3.0).

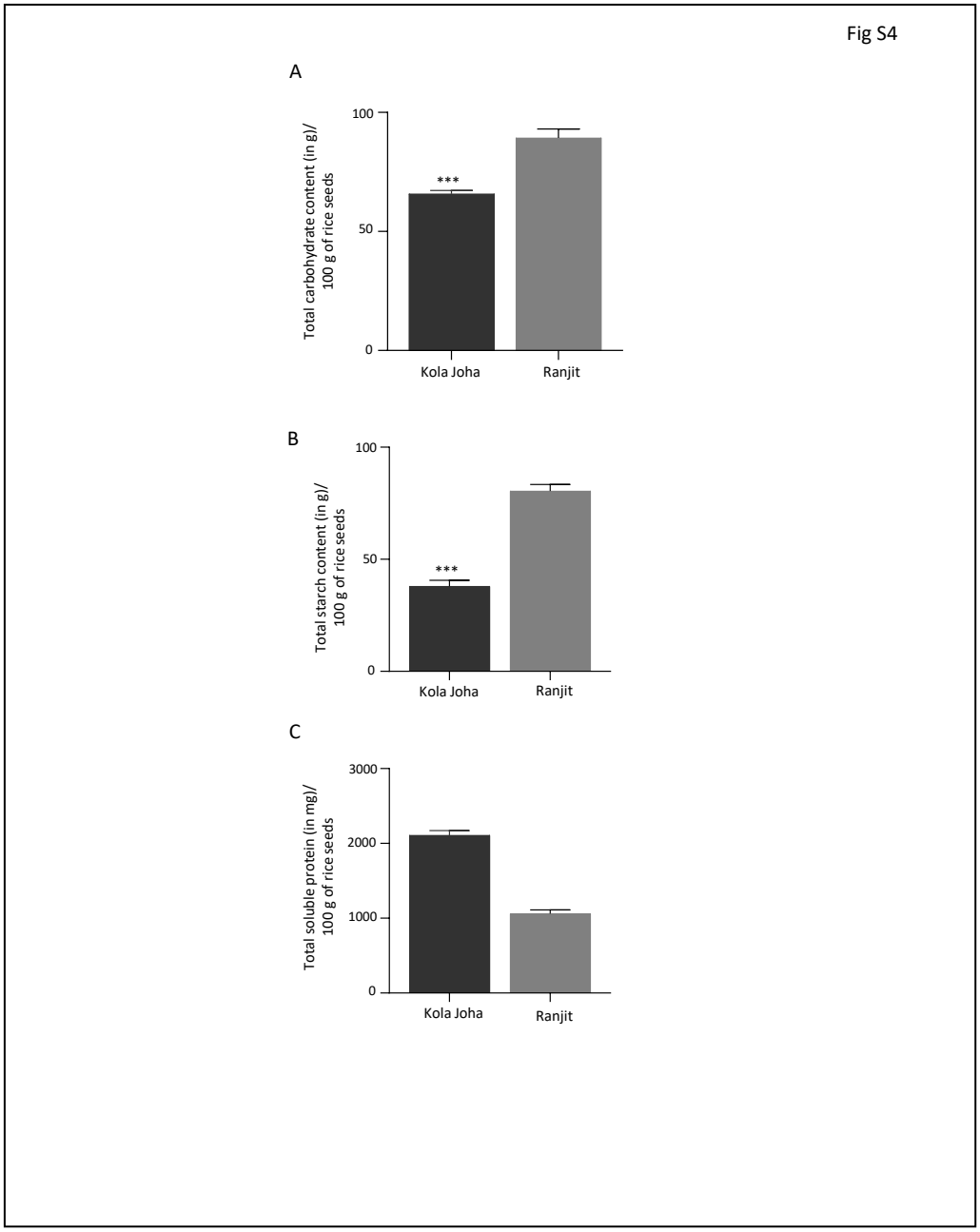

Fig. S4. Nutrient content analysis of scented rice Kola Joha and non-scented rice Ranjit. (A) Total carbohydrate was measured (in grams) per 100 g of rice seeds by Anthrone method; (B) Total starch content was measure (in grams) per 100 g of sample by Anthrone method; (C) Total soluble protein content per 100 g of rice seeds was measured (in mg) by Bradford method. Results are shown as mean ± SD (n = 3). ∗Statistically significant (P <0.05) compared to other sample was calculated by unpaired student t-test.

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