

1. Preliminary studies

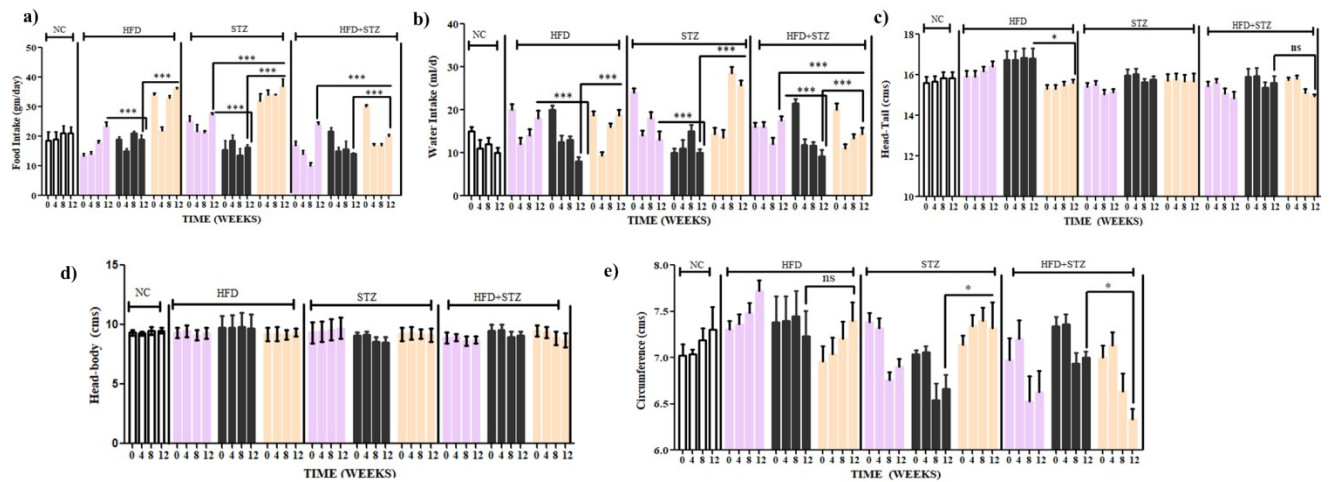
In the preliminary phase of our research, we meticulously standardized the streptozotocin (a compound toxic to pancreatic islet β cells) and insulin (a hormone released by islet β cells for blood glucose absorption) dose combination, in order to establish and manage a type 2 diabetic model, ensuring consistency, reliability, and providing a robust foundation for subsequent investigation phases. During the establishment of T2DM using STZ in normal pellet diet (NPD) with or without high fat diet (HFD) resulted in high mortality rate (**Supplementary table 1**). Mortality was also observed after STZ among the rats fed with BWC and WWC with or without HFD, while least mortality was observed in positive controls administered with 1U insulin, prompting the administration of insulin to prevent such occurrences. Subsequently 0.5 U insulin administration along with STZ in non-positive control treatment groups reduced mortality and was followed further.

Chapatti preparation: The anthocyanin-rich black and white wheat seeds were grinded to obtain whole wheat flour. Unfermented flat bread (chapattis) were prepared, dried, and then ground into a fine powder (Kumari et al., 2020).

Supplementary Table 1. Standardized the STZ and insulin dose combination to reduce mortality and increase reliability

	Animal Groups	No. of animals	STZ dose (mg/kg)	Mortality
Without insulin dosage				
1	NPD + STZ	6	55	5 (Higher mortality)
2	BWC + STZ	6	55	2

3	WWC + STZ	6	55	3
4	NPD + HFD + STZ	6	55	5 (Higher mortality)
5	BWC + HFD + STZ	6	55	3
6	WWC + HFD + STZ	6	55	4
	NPD + STZ + 1 U INSULIN (positive control)	6	55	1
	NPD + HFD + STZ + 1U INSULIN (Positive control)	6	55	0
After insulin dosage				
2	BWC + STZ + INSULIN (0.5U)	6	55	0
3	WWC + STZ + INSULIN (0.5U)	6	55	0
5	BWC + HFD + STZ + INSULIN (0.5U) (0.5U)	6	55	0
6	WWC + HFD + STZ + INSULIN (0.5U) (0.5U)	6	55	1



2. Supplementary Figures

2.2 Morphometric parameters

Supplementary Fig. 1 Continuous physical observation of rats during the 12-week study period.

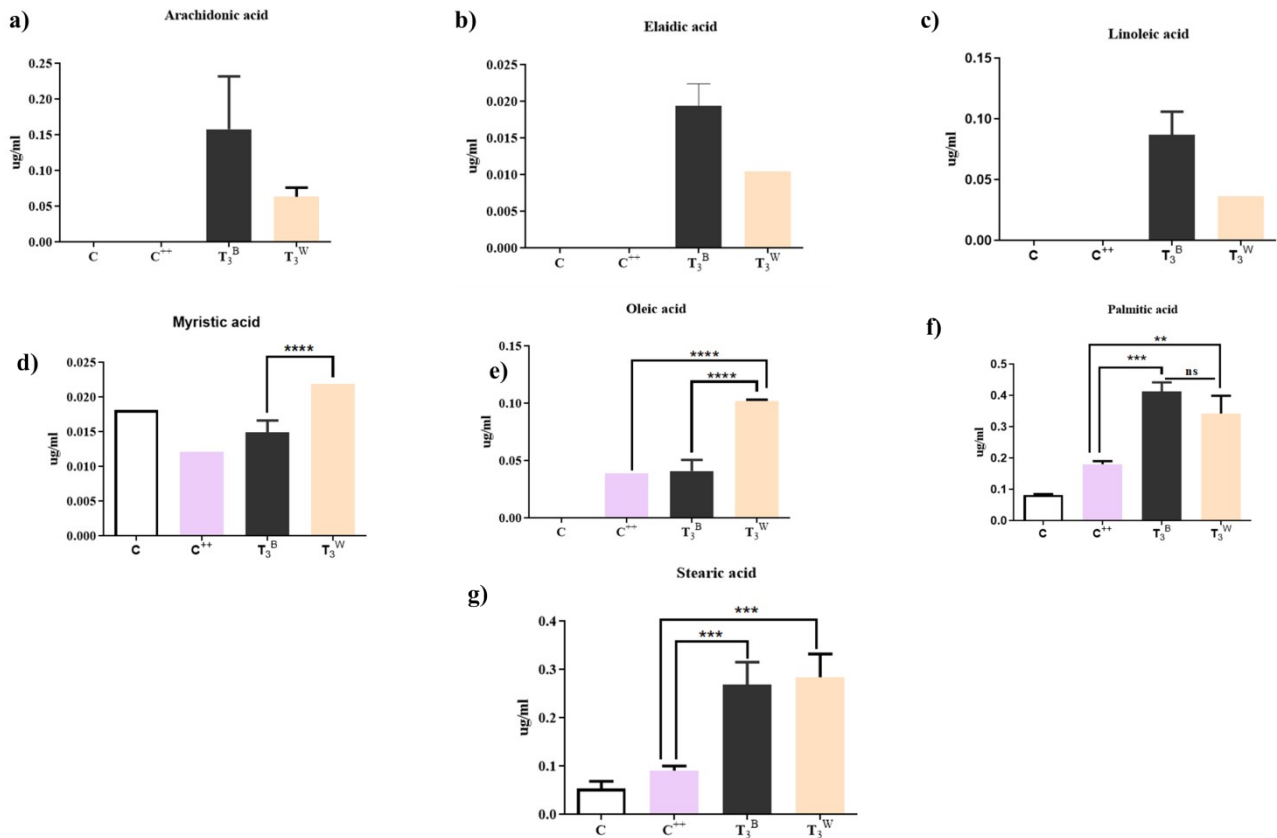
a, b Food and water intake were significantly improved in the black wheat control and type 2 diabetic groups compared to the white wheat groups

c, d, e Morphometric parameters, head-body, head-tail and circumference, were significantly improved at the 12th week of the experiment.

All values are expressed as mean \pm SD

Significance levels: * $p < 0.05$, ** $p < 0.01$, * $p < 0.001$**

Abbreviations: C (NPD+Control group), C⁻ (NPD+HFD), T₁^B (BWC+HFD), T₁^W (WWC+HFD), C⁺⁺ (NPD+STZ+INSULIN), T₂^B (BWC+STZ), T₂^W (BWC+STZ), C⁺⁺ (NPD+STZ+HFD+INSULIN), T₃^B (BWC+HFD+STZ), T₃^W (WWC+HFD+STZ)



Supplementary Fig.2 Anthocyanin rich colored wheat ameliorates the fatty acid composition at the 12 week intervention study.

a, c: Black wheat supplementation improves the omega-6 polyunsaturated (PUFA's) composition in blood as compared to the white wheat chapati in the T2DM rats.

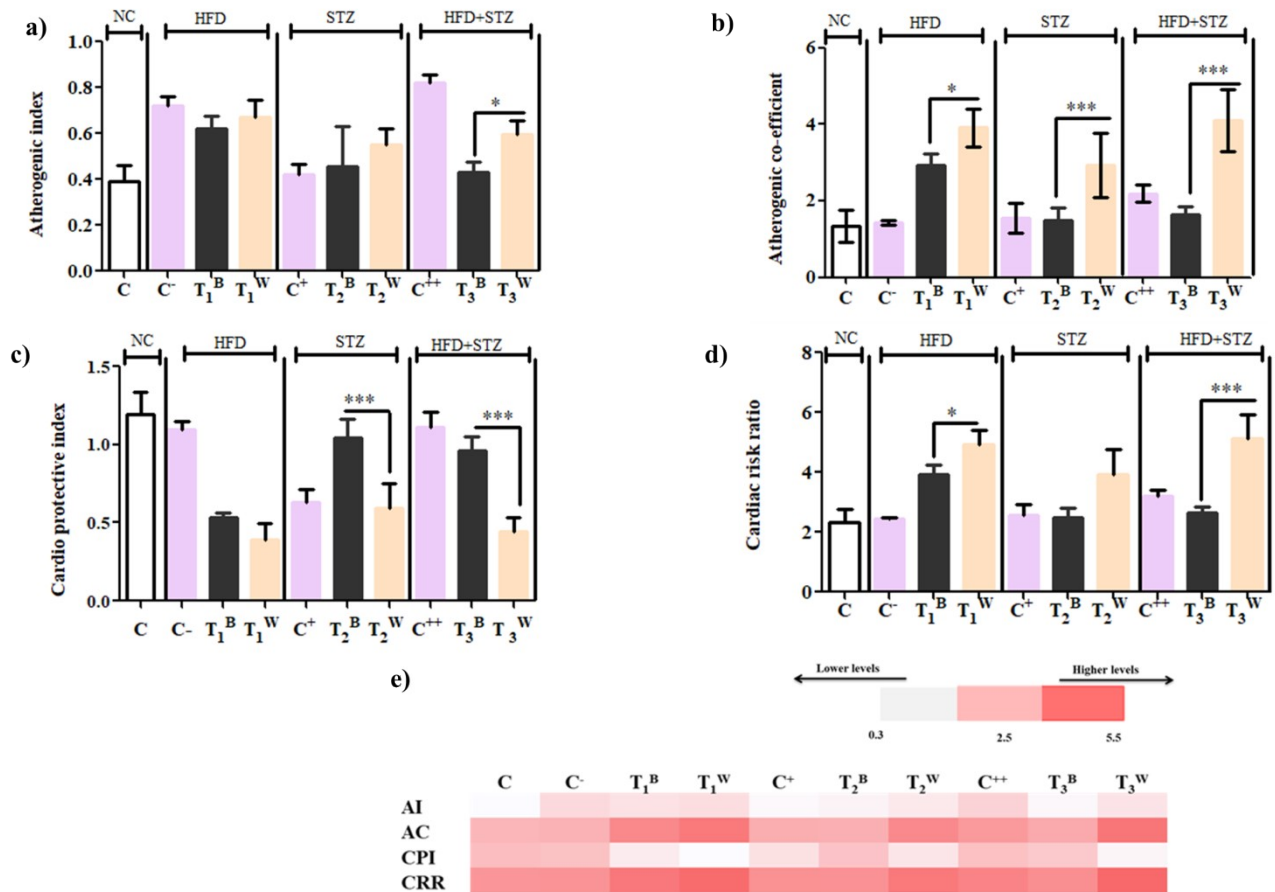
d, e: Monounsaturated fatty acids (MUFA's) and saturated fatty acid such as oleic , myristic acid were significantly higher in the white wheat T₃^W T2DM group.

f, g: No significant change was observed in the palmitic and stearic acid composition between the black wheat and white wheat T2DM groups.

All values are expressed as mean \pm SD

Significant level was denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$,

Abbreviations: C (NPD+Control group), C⁺⁺(NPD+STZ+HFD+INSULIN), T₃^B (BWC+HFD+STZ), T₃^W (WWC+HFD+STZ)



Supplementary Fig. 3 Calculation and evaluation of atherogenic and cardio protective indexes.

a, b, d: Black wheat attenuated the T2DM induced deterioration in atherogenicity by reducing the atherogenic index, atherogenic co-efficient and cardiac risk ratio

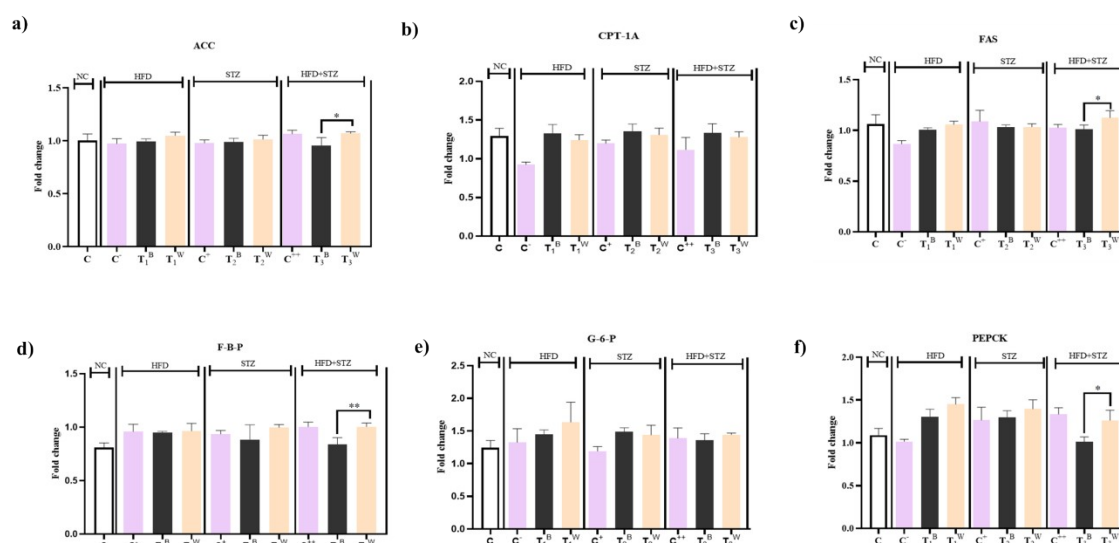
c. Black wheat ameliorates the Cardioprotective index

e: The heat map supporting all observations and indicating that T₃^B behaved similar to control NPD.

All values are expressed as mean ± SD

Significant level was denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Abbreviations: C (NPD+Control group), C⁻ (NPD+HFD), T₁^B (BWC+HFD), T₁^W (WWC+HFD), C⁺ (NPD+STZ+INSULIN), T₂^B (BWC+STZ), T₂^W (BWC+STZ), C⁺⁺ (NPD+STZ+HFD+INSULIN), T₃^B (BWC+HFD+STZ), T₃^W (WWC+HFD+STZ)



Supplementary Fig.4 Anthocyanin rich colored wheat ameliorated the fatty acid oxidation genes and gluconeogenesis pathway genes in the T2DM groups as compared to white wheat groups at the end of 12 weeks supplementation.

a, c, d, f Black wheat supplementation improves the ACC, FAS, F-B-P, and PEPCK expression in the T2DM black wheat chapati fed groups.

b, e There was no significant change observed in the expression of CPT-1A and G-6-P in overall groups of the study

All values are expressed as mean \pm SD

Significant level was denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Abbreviations: ACC- Acetyl-CoA carboxylase, CPT1- Carnitinepalmitoyltransferase-I, FAS- Fatty acid synthase, G-6-P - glucose 6 phosphatase, F-B-P- Fructose-1,6-bisphosphatase, PEPCK- Phosphoenolpyruvatecarboxykinase, C (NPD+Control group), C⁻ (NPD+HFD), T₁^B (BWC+HFD), T₁^W (WWC+HFD), C⁺ (NPD+STZ+INSULIN), T₂^B (BWC+STZ), T₂^W (BWC+STZ), C⁺⁺ (NPD+STZ+HFD+INSULIN), T₃^B (BWC+HFD+STZ), T₃^W (WWC+HFD+STZ)

Reference

** Kumari, A., Sharma, S., Sharma, N., Chunduri, V., Kapoor, P., Kaur, S., ... & Garg, M. (2020). Influence of biofortified colored wheats (purple, blue, black) on physicochemical, antioxidant and sensory characteristics of chapatti (Indian flatbread). *Molecules*, 25(21), 5071.

