Check for updates

*Original Research Article*

# Investigation of Ameliorative Effects of Black Rice and Purple Sweet Potato Extracts-rich Anthocyanins Against Animal Model of Insulin Resistance and Type 2 Diabetes

Pharmacognosy Magazine
22(3) 1066–1075, 2026
© The Author(s) 2025
Article reuse guidelines:
in.sagepub.com/journals-permissions-india
DOI: 10.1177/09731296251384042
journals.sagepub.com/home/phm
Sage

**Suhas Dhondibhau Tambe[1], P. Sivakami Sundari[1] and Geetha Kannoth Mukundan[1]**

## Abstract

**Background:** Nutraceuticals are popular because of a wide variety of biological actions, largely because of the contents present like alkaloids, glycosides, terpenoids, oils, tannins and several other phytochemicals. Anthocyanins are water-soluble pigments found in plants as secondary metabolites and can be of great therapeutic interest for the management of metabolic illnesses linked to diabetes and resistance related to insulin signalling.
**Objectives:** To figure out how nutraceuticals work for the treatment/improvement of inflammation-associated conditions developed due to a high high-calorie containing fatty diet and developed obesity, insulin signalling abnormalities and type 2 diabetes.
**Materials and Methods:** Intraperitoneal macrophages (IPMACs) were isolated from mice. These IPMACs were pretreated with extracts of black rice (BR) and purple sweet potato (PSP), followed by lipopolysaccharide (LPS) challenge to evaluate their effect on tumour necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6). Further, the inhibitory effect of pretreatment with BR and PSP was evaluated on the release of LPS-mediated TNF-α and IL-6 in C57BL/6 mice. In a separate experiment, extracts of BR and PSP were given orally to obese mice (a diet containing 60% high-fat diet) for 30 days to assess their impact on insulin resistance-mediated type 2 diabetes.
**Results and Discussion:** Extracts of BR and PSP significantly reduced cytokines like IL-6 and TNF-α in a dose-related manner in LPS-challenged macrophages. Treatment of diet-induced obesity mice with these extracts decreased fed blood glucose levels, supporting the hypothesis that reducing inflammation could help improve insulin resistance. Extracts of BR and PSP also attenuated peripheral insulin resistance after multiple days of treatment.
**Conclusion:** The results demonstrate that extracts of BR and PSP containing anthocyanins may be useful agents in improving inflammation-associated insulin resistance and type 2 diabetes.

## Keywords

Anthocyanins, insulin resistance, cytokines, glucose intolerance, type 2 diabetes

**Received** 18 February 2025; **revised** 18 May 2025; **accepted** 08 September 2025

## Introduction

Chronic disorders associated with reduced insulin sensitivity and associated obesity, type 2 diabetes (T2D), are evident due to a chronic increase in inflammation developed due to overnutrition of fat.

Adipocytes play a major role in the chronic inflammatory response by secretion of multiple chemokines, cytokines and adipokines (Sartipy & Loskutoff, 2003). Once the macrophages get activated in adipocytes, they act as a major trigger for the release of inflammatory cytokines and chemokines, leading to a continuous state of chronic inflammation, which interferes with insulin signalling, thereby inducing insulin resistance (Boden, 2008). This state of chronic inflammation, adipocyte hyperplasia and hypertrophy does not remain

[1]Department of Pharmacology, College of Pharmaceutical Sciences, Dayananda Sagar University, Bengaluru, Karnataka, India

**Corresponding author:**
P. Sivakami Sundari, Department of Pharmacognosy, College of Pharmaceutical Sciences, Dayananda Sagar University, Devarakaggalahalli, Harohalli, Kanakapura Road, Ramanagara, Bengaluru, Karnataka 562112, India.
E-mail: sivakami-sps@dsu.edu.in

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (http://www.creativecommons.org/licenses/by-nc/4.0/) which permits non-Commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).

restricted to the AT. These secreted cytokines, chemokines, also travel to other organs like the liver and skeletal muscle, and further contribute to the development of inflammation and insulin resistance (Larsen et al., 2007; McGonagle et al., 2008; Osborn et al., 2008).

Anthocyanins have been used for a long time as a nutraceutical, phytopharmaceutical, choleretic agent, appetite stimulant and treatment for various chronic ailments (Gonçalves et al., 2021).

It is reported that anthocyanins have mechanisms that can reduce insulin resistance, hyperglycaemia, inhibit gluconeogenesis and actions of carbohydrate-hydrolysing enzymes α-amylase and glucosidase and as such normalise glucose levels, improve insulin secretion and proliferation of β-cells of the pancreas (Güder et al., 2015; Noordin et al., 2019). Cornus fruits, containing high levels of anthocyanins, are used in Chinese prescriptions traditionally for the treatment of diabetes (Das et al., 2020; He & Giusti, 2010). Obese mice fed a meal rich in cyanidin-3-glucoside derived from purple maize experienced weight loss and a reduction in fat mass. The study shows that when these obese rats were treated with a purple corn diet, they showed a reduction in glucose levels and improvement in insulin, leptin and tumour necrosis factor-alpha (TNF-α) messenger ribonucleic acid (mRNA) levels (Tsuda et al., 2003). Research has demonstrated that anthocyanins reduce interleukin 6 (IL-6) levels and attenuate inflammatory responses in obese older individuals following a high-energy from fat meal challenge. Blackcurrant extracts (BCE), rich in anthocyanins, treatment significantly reduced IL-1β and IL-6 mRNA levels post lipopolysaccharide (LPS) challenge in the macrophage cell line RAW 264.7. Pretreatment of RAW 264.7 macrophages with BCE significantly reduced mRNA levels of IL-6, IL-1β and TNF-α (Lee & Lee, 2019). Black raspberry extract significantly reduced mRNA expression of pro-inflammatory genes in liver tissue, such as IL-1β, IL-6, cyclooxygenase-2 (COX-2) and nuclear factor-κB (NF-κB) in rats fed high-choline, high-fat diets (HFDs). Moreover, protein expression of COX-2 and NF-κB in liver tissue was also attenuated with black raspberry extract. Purple sweet potato anthocyanins (PSPA) treatment in a type 2 diabetes mellitus (T2DM) mouse model has shown reduced blood glucose levels, increased serum antioxidant enzymes and optimised the diversity and structure of the gut microbiota (Mi et al., 2024).

The effects of anthocyanin-rich sources were not investigated in detail. Therefore, the current study was performed to (a) comprehend the function of inflammation-associated insulin resistance in the high-fat diet-induced obesity (DIO) model of mice; and (b) ascertain whether extracts of purple sweet potato (PSP) and black rice (BR) are useful in reducing or attenuating the obesity, inflammation and T2D associated with insulin resistance in mice.

## Materials and Methods

### Experimental Designs and Protocols

#### Extraction of BR and PSP

Extraction of BR: 100 g of BR was coarsely powdered using a grinder (Preethi Steele Supreme 750), and the powdered rice was extracted with 500 mL of hydroethanol (80:20). The mixture was kept in a water bath, and the contents were refluxed for 3 h. The mixture was filtered with Whatman No. 1 filter paper, and the filtrate was then evaporated *in vacuo* to 100 mL and then transferred to an evaporating dish, where it was left to dry at room temperature. The dried sample (~4 g) was further pulverised into powder in a mortar and pestle.

Extraction of PSP: 100 g of skin was peeled and coarsely powdered using a grinder (Preethi Steele Supreme 750), and the powder was transferred to a 3-L round-bottom flask. It was extracted with 500 mL of hydroethanol (80:20), and the mixture was kept in a water bath, and the contents were refluxed for 3 h. The mixture was then filtered with Whatman No. 1 filter paper, and the filtrate was then evaporated *in vacuo* to 100 mL and then transferred to an evaporating dish, where it was left to dry at room temperature. The dried sample (~4 g) was further pulverised into powder in a mortar and pestle.

### Animals

6–9 weeks old male C57BL/6 mice weighing between 22 and 25 g were used in the experiment. All procedures were under the guidelines provided by the Committee for Control and Supervision of Experiments on Animals (CCSEA), India. Study design and procedures followed herein have been accepted by the Institutional Animal Ethics Committee (IAEC) of Eurofins Advinus Limited (Approval no.: Renewal_008_April-2021). All the animals were fed a low-fat diet (LFD) consisting of 10% calories from fat (D12450B; Research Diets Inc., USA) before the start of the experiment. Animals were then fed with a HFD consisting of 60% calories from fat (D12492; Research Diets Inc., USA) for the development of obesity (DIO model) (Singh & Pattnaik, 2024a).

### Proof of Concept Assay in Lipopolysaccharide-primed Peritoneal Macrophages Derived from C57BL/6 Mice

Male C57BL/6 mice were injected intraperitoneally with phosphate-buffered saline (PBS), and after 10–15 min, mice were anaesthetised with isoflurane anaesthesia and then sacrificed by cervical dislocation. The peritoneal cavity was cut open, and PBS in the cavity was aspirated using a syringe. This aspirated PBS was transferred to a Falcon tube and centrifuged at 6,000 RPM. Supernatant was discarded, and the pellet containing macrophages was

isolated. These macrophages were then cultured in Dulbecco's minimum essential medium (DMEM) containing 10% foetal bovine serum (FBS) and 10 mg/mL gentamicin at 37ºC in a humidified, 5% $CO_2$/95% air atmosphere. These macrophages were pretreated with different concentrations (0.3–6 mg/mL) of extracts of BR and PSP and then stimulated with 1 ng/mL of LPS. Then the macrophages were incubated overnight, and the supernatants were collected. These supernatants were used for the estimation of inflammatory cytokines, TNF-α and IL-6, using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, USA) according to the manufacturer's manual for the respective kit for the procedure.

### *Acute Effect of Extracts on Lipopolysaccharide-induced Cytokine Release in C57BL/6 Mice*

C57BL/6 mice weighing 22–25 g and 8–10 weeks old were used for the experiment. These mice were given different doses of BR and PSP extracts or a vehicle. Then, 3–4 h post-treatment, these mice were dosed by a single intraperitoneal injection of LPS at a 1 mg/kg dose as shown below:

- Vehicle (0.5% Methocel + 0.5% Tween 80), 10 mL/kg, PO + LPS, 1 mg/kg, IP
- Sweet potato extract, 30 mg/kg, PO + LPS, 1 mg/kg, IP
- Sweet potato extract, 100 mg/kg, PO + LPS, 1 mg/kg, IP
- Sweet potato extract, 300 mg/kg, PO + LPS, 1 mg/kg, IP
- BR extract, 30 mg/kg, PO + LPS, 1 mg/kg, IP
- BR extract, 100 mg/kg, PO + LPS, 1 mg/kg, IP
- BR extract, 300 mg/kg, PO + LPS, 1 mg/kg, IP

Blood samples were collected post-3–4 h of LPS treatment for the measurement of plasma IL-6 and TNF-α levels using commercially available ELISA kits (R&D Systems, USA) according to the manufacturer's manual for estimation.

### *Chronic In Vivo Animal Study*

C57BL/6 mice weighing 22–25 g and 8–10 weeks old were fed on LFD for the control group ($n = 6$) and with HFD for 12–16 weeks to develop diet-induced obese mice. Once the mice were obese, ad-lib fed blood glucose levels were monitored using a glucometer (AccuSure Simple®, MicroGene Diagnostic Systems Pvt., Ltd). Animals were then randomised according to blood glucose and body weight into various treatment groups ($n = 6$) as shown in Table 1.

All the animals were treated once daily in the morning, around 9 AM to 10 AM, with their respective treatment groups for a period of 30 days. Body weight, ad-lib fed blood glucose and food intake were monitored before the dose and on days 7, 14, 21 and 28. On day 30, food was removed, and animals fasted for 6–8 h, and blood samples were collected under anaesthesia by cardiac puncture. Blood samples were centrifuged, and plasma samples were separated for the estimation of triglycerides (TGs) and total cholesterol with commercially available diagnostic kits from Wako Pure Chemicals (Maedler et al., 2002; Vandanmagsa et al., 2011; Vatier et al., 2012).

### *Insulin and Glucose Tolerance Test for the Assessment of Insulin Sensitivity*

Animals underwent an insulin tolerance test (ITT) and an oral glucose tolerance test (OGTT) on day 21 and day 28, respectively. For ITT, 0.75 IU/kg of human insulin (Actrapid) was injected intraperitoneally in the fed state. For OGTT, animals were fasted for 14 h and blood glucose levels were monitored. Then, animals were orally dosed with 2 g/kg of glucose (Masters et al., 2010). For all the measurements, the tail snip technique was used to get blood samples. Blood glucose levels were monitored with the help of a glucometer (AccuSure Simple®, MicroGene Diagnostic Systems Pvt., Ltd) before the treatment with extracts (pre-treatment) and 1 h posttreatment as baseline, as 0 min (pre-dose of insulin or glucose) and at 15, 30, 60, 120 and 180 min post-dosing of insulin or glucose, respectively. Area under curve (AUC) was calculated for glucose from 0 to 180 min (Stienstra et al., 2010).

### *Statistical Analysis*

All the values in graphs are presented as mean ± SEM. All data generated in this study were analysed using one-way analysis of variance (ANOVA) at $p < .05$, and *post hoc* comparison of Dunnett with control was used.

**Table 1.** Different Animal Groups and Treatments Administered in Chronic *In Vivo* Animal Study.

| Study Groups ($n = 6$) | Diet Fed | Treatment Received |
|---|---|---|
| LFD + vehicle | LFD (10%) | Vehicle (0.5%–1% Tween 80 + 0.5% CMC), PO, q.d. |
| HFD + vehicle | HFD (60%) | Vehicle (0.5% Methocel + 0.5% Tween 80), PO, q.d. |
| HFD + black rice | | Black rice extract, 100 mg/kg, PO, q.d. |
| HFD + purple sweet potato | | Sweet potato extract, 100 mg/kg, PO, q.d. |
| HFD + pioglitazone | | Pioglitazone (30 mg/kg), PO, q.d. |

**Note:** HFD: High-fat diet; LFD: Low-fat diet.

## Results

### BR and PSP Extracts Inhibit LPS-mediated TNF-α and IL-6 Levels in Intraperitoneal Macrophages (IPMACs)

In order to check if BR and PSP extracts have an inhibitory effect on inflammatory cytokines, we analysed TNF-α and IL-6 levels by ELISA. Resident peritoneal macrophages were incubated with either vehicle or various concentrations of BR and PSP extracts. Then these macrophages were stimulated with a final concentration of 1 ng/mL of LPS. Both IL-6 and TNF-α levels were markedly elevated in LPS-primed IPMACs. Both BR and PSP extracts showed significant inhibition of IL-6 and TNF-α in LPS-primed macrophages in a dose-related manner (Figure 1A, 1B, 1C and 1D). This data suggests that BR and PSP extracts containing anthocyanins have a potential inhibitory effect on inflammatory cytokines.

### BR and PSP Extracts Inhibit LPS-mediated IL-6 and TNF-α Levels in C57BL/6 Mice

To check the translation of observed effects in isolated IPMACs under *in vitro* conditions to *in vivo* conditions, BR

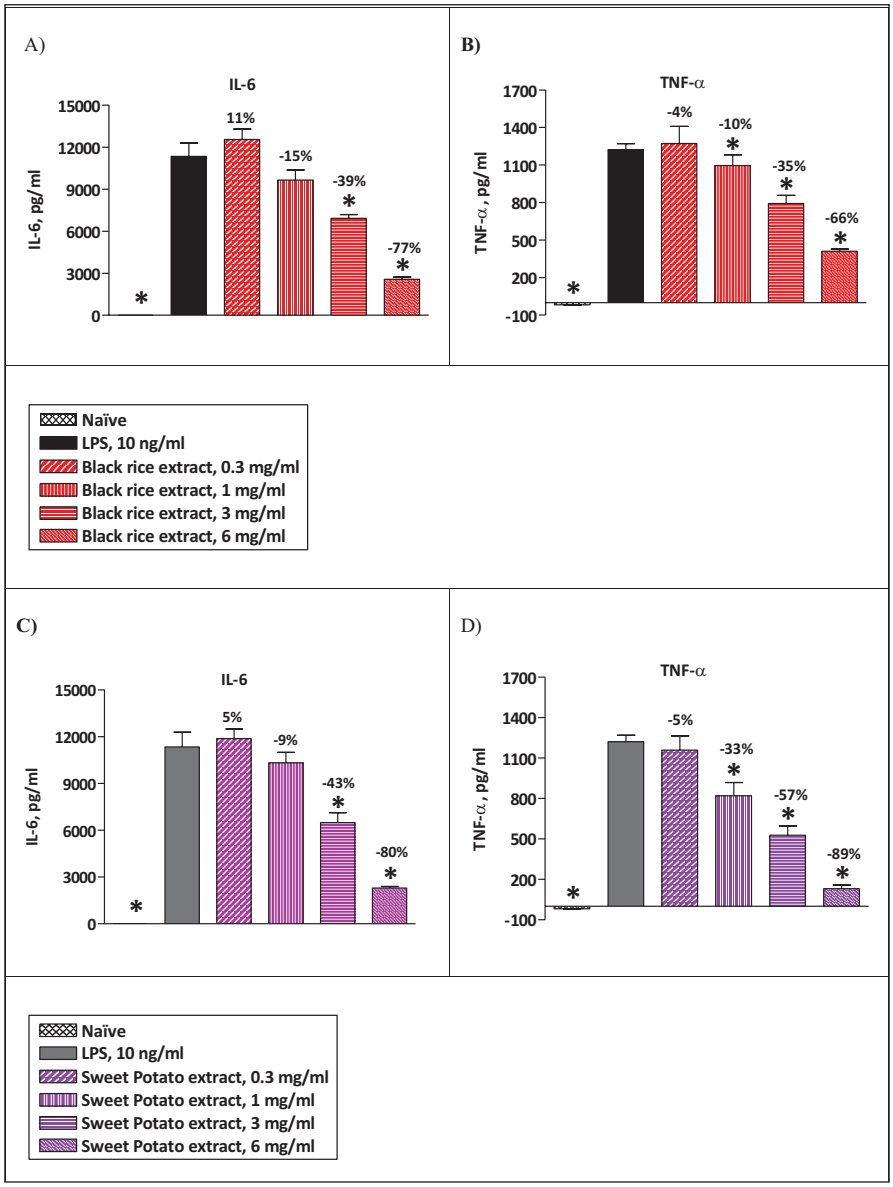



**Figure 1.** Effect of Black Rice and Purple Sweet Potato Extracts (3–300 mg/mL) on (A and C) IL-6 and (B and D) TNF-α Levels, Respectively, in LPS-stimulated Mouse Peritoneal Macrophages. *$p < .05$ Compared to LPS. Black Rice and Purple Sweet Potato Extracts Showed Significant and Dose-dependent Reduction in TNF-α and IL-6 Levels as Compared to Vehicle Treatment.

**Note:** IL-6: **Interleukin 6**; LPS: Lipopolysaccharide; TNF-α: Tumour necrosis factor-alpha.

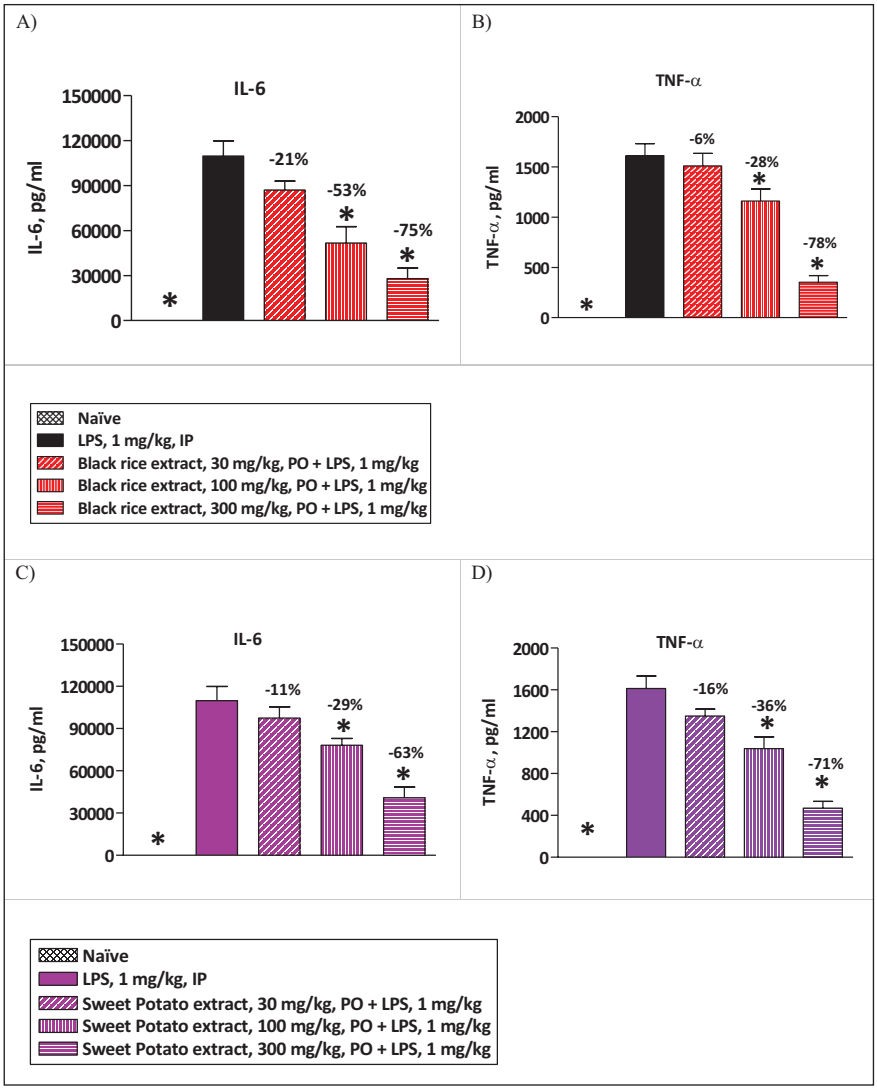



**Figure 2.** The Effect of Black Rice and Purple Sweet Potato Extracts (30–300 mg/kg) on LPS-induced Changes in Plasma Levels of (A and C) IL-6 and (B and D) TNF-α Levels, Respectively, in C57BL/6 Mice. Data is Shown as Mean ± SEM *$p < .05$ Compared to LPS-treated Mice. Black Rice and Purple Sweet Potato Extracts Showed Significant and Dose-dependent Reduction in PLASMA LEVELS of IL-6 and TNF-α Levels as Compared to LPS-challenged Treated Mice.

**Note:** IL-6: Interleukin 6; LPS: Lipopolysaccharide; TNF-α: Tumour necrosis factor-alpha.

and PSP extracts were administered orally to C57BL/6 mice, followed by LPS treatment. Both TNF-α and IL-6 levels were significantly higher in LPS-treated mice as compared to saline-treated mice. Pretreatment of these mice with BR and PST extracts significantly reduced plasma TNF-α and IL-6 levels in comparison to LPS-challenged mice in a dose-dependent manner (Figure 2A, 2B, 2C and 2D). This data suggests that BR and PSP extracts containing anthocyanins show inhibition of inflammatory cytokine release when stimulated by inflammatory mediators like LPS under *in vivo* conditions.

## *Effect of BR and PSP Extracts Treatment on Fed Blood Glucose, Body Weight and Feed Intake in DIO Mice*

To determine the result of BR and PSP extracts on obesity-induced insulin resistance, mice were fed with either LFD or HFD for 14–16 weeks. Blood glucose, body weight and feed intake were monitored on days 1, 7, 14 and 21 during the course of treatment (Table 2). Daily treatment at 100 mg/kg daily doses of BR and PSP extracts significantly improved fed blood glucose levels on day 14 and day 21 of treatment in

**Table 2.** Changes in Blood Glucose Levels on Various Days of Treatment.

| | Fed Blood Glucose (mg/dL) | | | |
|---|---|---|---|---|
| **Groups** | **Day 1** | **Day 7** | **Day 14** | **Day 21** |
| LFD + vehicle | 117.5 ± 3.7 | 118.7 ± 4.2 | 127 ± 3.7 | 121.3 ± 2.9 |
| HFD + vehicle | 182.0 ± 4.9 | 178.3 ± 4.0 | 180.2 ± 4.3 | 179.8 ± 5.2 |
| HFD + black rice | 181.0 ± 5.5 | 161.7 ± 5.6 | 143.8 ± 2.0* | 136.8 ± 4.2* |
| HFD + purple sweet potato | 180.5 ± 4.7 | 155.8 ± 4.6 | 151.3 ± 3.7* | 145.3 ± 5.9* |
| HFD + pioglitazone | 180.8 ± 3.3 | 150.2 ± 5.9* | 132.8 ± 4.0* | 132.2 ± 5.2* |

**Notes:** *$p < .05$, compared with HFD + vehicle group. HFD: High-fat diet; LFD: Low-fat diet.

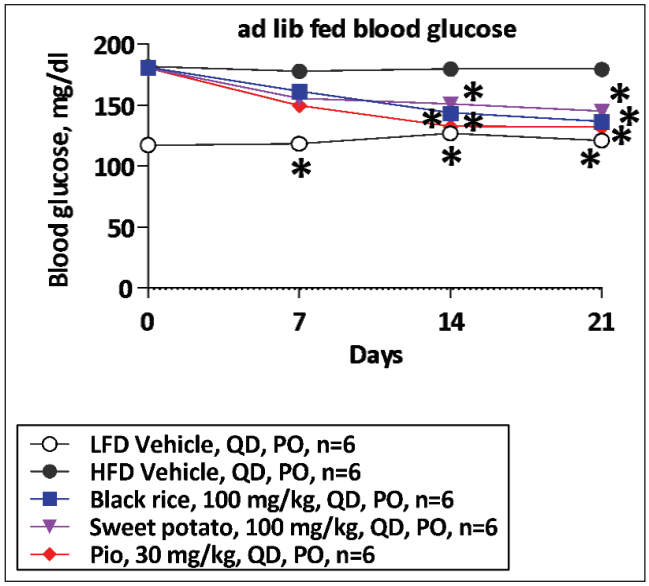



**Figure 3.** Effect of Black Rice and Purple Sweet Potato Extracts (100 mg/kg) and Pioglitazone (30 mg/kg) on *Ad Libitum* Fed Blood Glucose ($n = 6$). Data is Shown as Mean ± SEM. *$p < .05$ Compared to Vehicle Treatment. Black Rice and Purple Sweet Potato Extracts and Pioglitazone Showed Significant Reduction in Ad-lib Fed Blood Glucose as Compared to Vehicle Treatment on Day 14 and Day 21.

comparison to control animals treated with vehicle. The reference standard treatment with pioglitazone (30 mg/kg) also revealed a remarkable improvement in fed glucose levels as compared to animals treated with vehicle (Figure 3). Body weight increased linearly in mice fed the HFD as compared to LFD controls. Treatment of obese mice with BR and PSP extracts and pioglitazone had no significant effect on body weight and feed intake.

## *Effect of BR and PSP Extracts on Intraperitoneal Insulin and Glucose Tolerance Tests in DIO Mice*

Long-term feeding of a high high-calorie diet led to a remarkable increase in adipocyte weight, and accumulation of free fatty acids and TGs in adipocytes, liver and muscles, thereby increasing insulin resistance or reducing insulin sensitivity (Sartipy & Loskutoff, 2003; Singh & Pattnaik, 2024b). There is also chronic inflammation in these metabolically important tissues, with significant increases in inflammatory cytokines like IL-6 and TNF-α levels in circulation. Therefore, our present study focuses on whether treatment of HFD-fed mice with BR and PSP extracts reduces insulin resistance and thereby improves insulin sensitivity after chronic treatment. Interestingly, during the ITT, our results also show a significant decrease in insulin sensitivity in HFD-fed mice compared to that of LFD-fed mice, as seen from less reduction in plasma glucose levels post-insulin challenge. Chronic treatment of mice with BR and PSP extracts showed lowered blood glucose levels, and AUC glucose during insulin injection testing (IIT) shows a considerable improvement in insulin sensitivity when compared to the vehicle treatment. This indicates the insulin-sensitising effect of these extracts. Pioglitazone, a very well-known insulin sensitiser, showed a remarkable improvement in insulin resistance in mice after chronic treatment as compared to vehicle control animals (Figure 4).

BR and PSP extracts treated animals showed significant reductions in glucose levels during OGTT at various time points and AUC blood glucose as compared to vehicle-treated groups, indicating the improvement in glucose intolerance. Pioglitazone treatment also lowered blood glucose levels significantly in comparison with vehicle-treatment group (Figure 5A and 5B).

## *Effect of BR and PSP Extracts on Adipose Tissue-derived Inflammatory Cytokines*

To inquire if BR and PSP extracts inhibit pro-inflammatory cytokines secreted in obesity, adipose tissues were gathered at the study's conclusion, and IL-6 and TNF-α levels were measured using ELISA kits. In the obesity condition, major cytokines such as IL-6 and TNF-α were detected at elevated levels in homogenised adipose tissue. IL-6 and TNF-α are also produced in conditions like HFD-mediated obesity and insulin resistance associated with obesity. BR and PSP extracts did not show statistically significant changes in any of these cytokines in adipose tissue homogenates, whereas pioglitazone (30 mg/kg) showed a significant reduction compared to vehicle-treated mice.

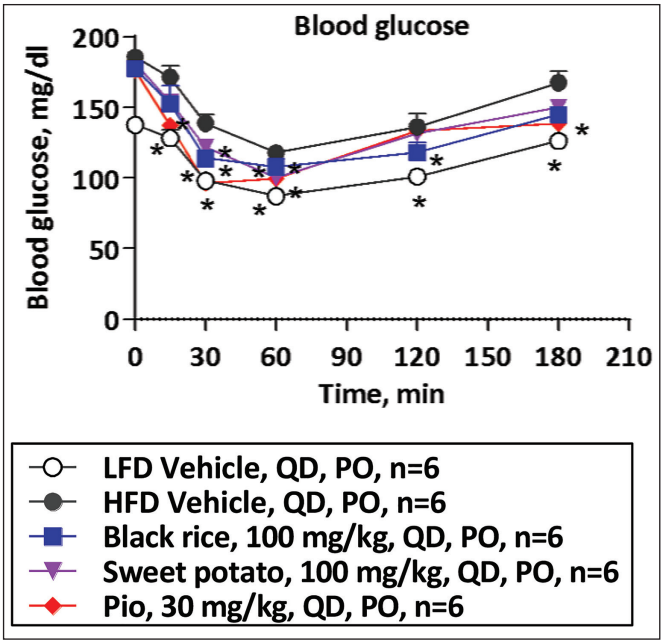



**Figure 4.** Effect of Effect of Black Rice and Purple Sweet Potato Extracts (100 mg/kg) and Pioglitazone (30 mg/kg) Treatment on Insulin Sensitivity. Data is Shown as Mean ± SEM *$p$ < .05 Compared to Vehicle Treatment, One-way ANOVA Followed by Dunnett's Multiple Comparison Test, *$p$ < .05 Compared to Vehicle Treatment. The Effect of Black Rice and Purple Sweet Potato Extracts Treatment Showed Moderate Improvement in Insulin Sensitivity During IIT as Seen from Lowered Blood Glucose as Compared to Vehicle Treatment.

**Note:** ANOVA: Analysis of variance; IIT: Insulin injection testing.

Although BR and PSP extracts showed significant and 100 mg/kg showed no significant cytokines inhibition in adipose tissues of DIO mice might be due to low dose and might have been a cogent inhibition at its higher doses.

### *Effect on Treatment on Plasma TGs and Total Cholesterol Levels in DIO Mice*

Total cholesterol and TG levels in plasma were high in HFD-induced obese mice as compared to their lead control LFD-fed C57BL/6J mice (Figure 6A and 6B). Chronic treatment for 30 days with BR and PSP extracts (100 mg/kg) and pioglitazone (30 mg/kg), significantly reduced plasma levels of TGs in contrast to animals treated in vehicles. Reduction in elevated total cholesterol in HFD-fed mice was not affected by treatment with BR and PSP extracts, as well as pioglitazone treatment.

## Discussion

Pro-inflammatory cytokines production and immune cells infiltration are contributed by obesity and related decrease in insulin sensitivity is ultimately due to chronic, low inflammation. In this condition, pro-inflammatory cytokines like TNF-α, IL-1β and IL-6 impart insulin resistance by interfering with insulin signalling and initiate inflammatory response and related insulin resistance. We studied the effect of chronic treatment with BR and PSP extracts, which contain anthocyanins, against a 60% fat-containing diet fed to mice to understand the role of pro-inflammatory cytokines in insulin resistance conditions following HFD feeding. *In vitro* target engagement assays were performed to verify if these extracts can inhibit or reduce LPS-challenged cytokine production. In these studies, both the extracts significantly inhibited IL-6 and TNF-α release in a dose-related manner in LPS-stimulated IPMACs. This data suggests that BR and PSP extracts have a very significant anti-inflammatory activity, which could be acknowledged by their mechanism of anti-inflammatory activity and reduction of associated secretion of pro-inflammatory cytokines. Further, pretreatment of normal C57BL/6 mice with these extracts significantly reduced LPS-induced cytokine production, indicating the translation of effect from *in vitro* to *in vivo* conditions and supporting our hypothesis that these extracts have anti-inflammatory properties.

Further to this, we first report that treatment of diet-induced obese mice with BR and PSP extracts reduced glucose intolerance, insulin resistance and obesity-associated inflammation. Additionally, plasma levels of TGs were remarkably reduced in mice treated with these extracts; however, there was no effect on total cholesterol levels. In total, the current study results support the hypothesis that low-grade chronic inflammation is closely associated with obesity and insulin resistance, and anti-inflammatory treatment could be effective in improving insulin sensitivity. In our study, BR and PSP extracts improved obesity-induced insulin resistance in DIO mice.

Type 2 diabetic patients with obesity show a reduction in inflammation and improved glucose homeostasis when treated with various herbal extracts containing anthocyanins. Therefore, we also evaluated the effect of the extracts on random blood glucose in DIO mice and found that there was a significant reduction in ad-lib fed blood glucose from day 14, over the course of treatment, as compared to the vehicle treatment group.

ITT and OGTT were performed to figure out the mechanism of anthocyanins in improving insulin and glucose homeostasis in HFD-fed obese. Mice treated with BR and PSP extracts had a moderate improvement in insulin sensitivity during IIT as seen by decreasing blood glucose levels in comparison to vehicle treatment, despite being fed an unhealthy diet. Blood glucose levels of BR and PSP extracts treated mice were significantly reduced during the OGTT study. Resulting in a reduction in chronic inflammation leads to improvement in insulin sensitivity, thereby improvement in blood glucose homeostasis, which supports our hypothesis. But in the case of feed intake and body weight, they were not

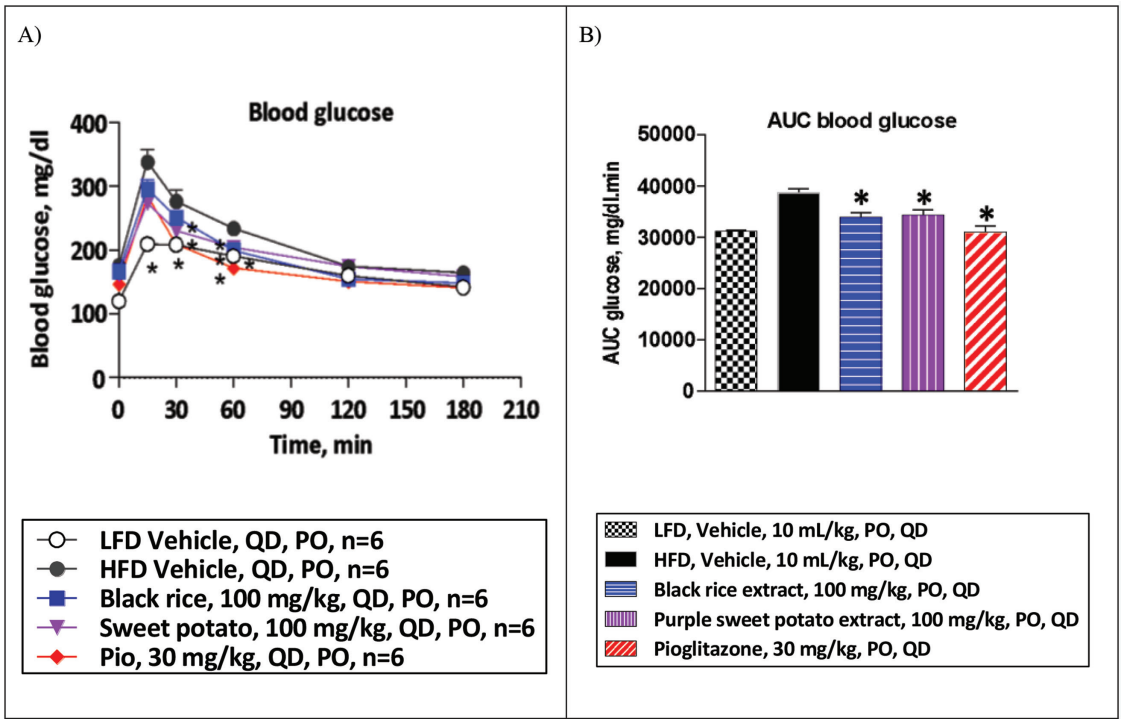



**Figure 5.** Effect of Black Rice and Purple Sweet Potato Extracts (100 mg/kg) and Pioglitazone (30 mg/kg) Treatments on Glucose Tolerance. Data is Shown as Mean ± SEM *$p < .05$ Compared to Vehicle, One-way ANOVA Followed by Dunnett's Multiple Comparison Test. Both Black Rice and Purple Sweet Potato Extracts and Pioglitazone Treatments Showed Significant Reduction in (A) Blood Glucose and (B) AUC Blood Glucose as Compared to Their Corresponding Vehicle-treated Groups on Day 28.

**Note:** ANOVA: Analysis of variance; AUC: Area under curve.

well correlated and did not show a significant effect in DIO mice.

According to the studies reported earlier, cause for β-cell dysfunction in the pancreas, pathogenesis of T2D, obesity-mediated inflammation and further resultant insulin resistance, are pro-inflammatory cytokines, IL-6 and TNF-α. In our study, *in vitro* and *in vivo* effects of these cytokines were evaluated for these extracts. It is observed that pro-inflammatory cytokines were released in significant quantities in adipose tissue homogenates. In the present study, BR and PSP extracts enervated IL-6 and TNF-α levels in LPS-challenged peritoneal macrophages but failed to cogently inhibit these cytokines in adipose tissue homogenates. This may be due to the shorter duration of treatment in the present study, and it might inhibit these pro-inflammatory cytokines from longer duration treatment with higher doses, which is not tested in the present study.

Production of cytokines regulates lipid metabolism and glucose homeostasis. An induction of lipoprotein lipase activity leading to hypertriglyceridemia is achieved by affecting lipid metabolism through the secretion of IL-6.

Given this, we examined the effect of treatment with BR and PSP extracts on TG and total cholesterol levels in plasma from DIO mice. Mice fed an HFD exhibited statistically higher plasma levels of total cholesterol and TGs compared to their lean control. Following 30 days of treatment with BR and PSP extracts and pioglitazone (30 mg/kg), plasma levels of TG were significantly decreased, which could be due to reduced entry of TG into the plasma as chylomicrons or very low-density lipoproteins (VLDL) or by increased clearance out of the plasma. This shows that a reduction in chronic inflammation also leads to a reduction in DIO-associated high TG, which is a cardiovascular risk factor and a marker for abnormal metabolic health.

To conclude, our data reveal that BR and PSP extracts inhibit inflammation, reduce obesity and improve insulin resistance in a mouse model of HFD-induced obesity. BR and PSP extracts significantly lowered random blood glucose levels and improved conditions like insulin resistance and glucose intolerance. BR and PSP extracts were capable of significantly inhibiting the release of inflammatory cytokines in C57BL/6 mice and LPS-induced peritoneal macrophages. However, the present study defects the downstream mechanistic studies to understand the downstream pathway in insulin signalling, like phosphoinositide 3-kinases (PI3K)/AKT, inflammation signalling for NF-κB in metabolically active tissues like liver and white adipose tissue, and further mRNA level or protein determination by western blot studies.

Collectively, our findings support the hypothesis that long-term treatment with anthocyanin-rich extracts of BR and PSP

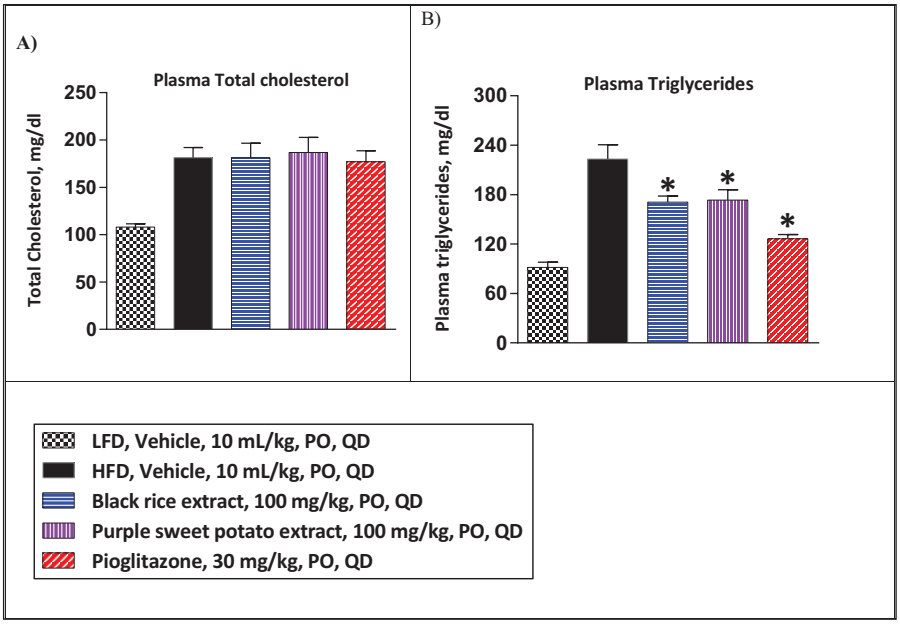



**Figure 6.** Effect of Black Rice and Purple Sweet Potato Extracts (100 mg/kg) and Pioglitazone (30 mg/kg) Treatments on Plasma (A) Total Cholesterol (TC) and (B) Triglyceride (TG) Levels. Data is Shown as Mean ± SEM *$p < .05$ Compared to Vehicle Treatment. One-way ANOVA Followed by Dunnett's Multiple Comparison Test. Black Rice and Purple Sweet Potato Extracts and Pioglitazone (30 mg/kg) Treatment Showed Significant Reduction in Plasma TG But Not TC Levels as Compared to Vehicle Treatment at Takedown on Day 30.

**Note:** ANOVA: Analysis of variance.

could be useful agents in improving inflammation associated with insulin resistance and T2D.

## Conclusion

Extracts of BR and PSP containing large amounts of anthocyanins showed dose-dependent reduction in inflammatory cytokines in isolated peritoneal macrophages. This effect was further strengthened when LPS included cytokines, which were significantly reduced with pretreatment in C57BL/6 mice. Further, there was translation of this anti-inflammatory effect in a chronic HFD-induced mouse model of T2D, wherein treatment with BR and PSP extracts improved insulin sensitivity and normalised blood glucose levels. Further long-term studies at higher doses in other animal models of T2D shall be performed to elucidate their usefulness in the development of pharmacotherapy for treatment in humans.

### Abbreviations

CPCSEA: Committee for the purpose of control and supervision of experiments on animals; IL-6: Interleukin-6; LPS: Lipopolysaccharide; TG: Triglyceride; TNF-α: Tumour necrosis factor-alpha.

### Authors' Contributions

Suhas Tambe was responsible for carrying out the research work and writing this article. P. Sivakami Sundari and Geetha Kannoth Mukundan were responsible for selecting the concept of research work as well as guiding and analysing the research outcomes.

### Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

### Ethical Approval

All the animal studies were approved by the Institutional Animal Ethical Committee with the approval number (Approval no.: Renewal_008_April-2021).

### Funding

The authors received no financial support for the research, authorship and/or publication of this article.

### Informed Consent

NA.

### ORCID iD

Suhas Dhondibhau Tambe https://orcid.org/0000-0002-5342-3926

### References

Boden, G. (2008). Ceramide: A contributor to insulin resistance or an innocent bystander? *Diabetologia*, *51*(7), 1095–1096. https://doi.org/10.1007/s00125-008-1015-y

Das, A., Geetha, K. M., & Hazarika, I. (2020). Contemporary updates on the physiology of glucagon like peptide-1 and its agonist to treat type 2 diabetes mellitus. *International Journal of Peptide Research and Therapeutics*, *26*(3), 1211–1221. https://doi.org/10.1007/s10989-019-09927-y

Gonçalves, A. C., Nunes, A. R., Falcão, A., Alves, G., & Silva, L. R. (2021). Dietary effects of anthocyanins in human health: A comprehensive review. *Pharmaceuticals*, *14*(7), Article 690. https://doi.org/10.3390/ph14070690

Güder, A., Gür, M., & Engin, M. S. (2015). Antidiabetic and antioxidant properties of bilberry (*Vaccinium myrtillus* Linn.) fruit and their chemical composition. *Journal of Agricultural Science and Technology*, *17*, 401–414.

He, J., & Giusti, M. M. (2010). Anthocyanins: Natural colorants with health-promoting properties. *Annual Review of Food Science and Technology*, *1*, 163–187. https://doi.org/10.1146/annurev.food.080708.100754

Larsen, C. M., Faulenbach, M., Vaag, A., Vølund, A., Ehses, J. A., Seifert, B., Mandrup-Poulsen, T., & Donath, M. Y. (2007). Interleukin-1-receptor antagonist in type 2 diabetes mellitus. *The New England Journal of Medicine*, *356*(15), 1517–1526. https://doi.org/10.1056/NEJMoa065213

Lee, Y., & Lee, J.-Y. (2019, May). Blackcurrant (*Ribes nigrum*) extract exerts an anti-inflammatory action by modulating macrophage phenotypes. *Nutrients*, *11*(5), Article 975. https://doi.org/10.3390/nu11050975

Maedler, K., Sergeev, P., Ris, F., Oberholzer, J., Joller-Jemelka, H. I., Spinas, G. A., Kaiser, N., Halban, P. A., & Donath, M. Y. (2002). Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets. *The Journal of Clinical Investigation*, *110*(6), 851–860. https://doi.org/10.1172/JCI15318

Masters, S. L., Dunne, A., Subramanian, S. L., Hull, R. L., Tannahill, G. M., Sharp, F. A., Becker, C., Franchi, L., Yoshihara, E., Chen, Z., Mullooly, N., Mielke, L. A., Harris, J., Coll, R. C., Mills, K. H. G., Mok, K. H., Newsholme, P., Nuñez, G., Yodoi, J., … O'Neill, L. A. J. (2010). Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1β in type 2 diabetes. *Nature Immunology*, *11*(10), 897–904. https://doi.org/10.1038/ni.1935

McGonagle, D., Tan, A. L., Madden, J., Emery, P., & McDermott, M. F. (2008). Successful treatment of resistant pseudogout with anakinra. *Arthritis and Rheumatism*, *58*(2), 631–633. https://doi.org/10.1002/art.23119

Mi, W., Hu, Z., Zhao, S., Wang, W., Lian, W., Lu, P., & Shi, T. (2024, May 23). Purple sweet potato anthocyanins normalize the blood glucose concentration and restore the gut microbiota in mice with type 2 diabetes mellitus. *Heliyon*, *10*(11), Article e31784. https://doi.org/10.1016/j.heliyon.2024.e31784

Noordin, L., Wan Mohamad Noor, W. N. I., Safuan, S., & Wan Ahmad, W. A. N. (2019). Therapeutic effects of anthocyanin-rich Hibiscus sabdariffa L. extract on body mass index, lipid profile and fatty liver in obese-hypercholesterolaemic rat model. *International Journal of Basic and Clinical Pharmacology*, *9*(1), 1–7. https://doi.org/10.18203/2319-2003.ijbcp20195759

Osborn, O., Brownell, S. E., Sanchez-Alavez, M., Salomon, D., Gram, H., & Bartfai, T. (2008). Treatment with an interleukin 1 beta antibody improves glycemic control in diet-induced obesity. *Cytokine*, *44*(1), 141–148. https://doi.org/10.1016/j.cyto.2008.07.004

Sartipy, P., & Loskutoff, D. J. (2003). Expression profiling identifies genes that continue to respond to insulin in adipocytes made insulin-resistant by treatment with tumor necrosis factor-alpha. *Journal of Biological Chemistry*, *278*(52), 52298–52306. https://doi.org/10.1074/jbc.M306922200

Singh, R. P., & Pattnaik, A. K. (2023). Anti-obesity potential of bioactive guided fractions of *Annona squamosa* Linn. *3 Biotech*, *13*(6), 171. https://doi.org/10.1007/s13205-023-03603-1

Singh, R. P., & Pattnaik, A. K. (2024a). Elucidating the anti-obesity potential of bioactive fractions of *Kalanchoe pinnata* (Lam.) leaves extract using a combination of *in vitro*, *in vivo* and *in silico* methods along with characterisation of lead compounds through an HPTLC MS-MSN analytical study. *Natural Product Research*, 1–6. https://doi.org/10.1080/14786419.2024.2344183

Singh, R. P., & Pattnaik, A. K. (2024b). Scientific insights into hyperlipidemia mitigation: A profound examination of isolated bioactive fractions of *Kalanchoe pinnata* (Lam.) leaves and their therapeutic implications using *in vitro*, *in vivo*, and *in silico* study from the characterized compounds using HPTLC MS/MSN analysis. *Pharmacognosy Magazine*, *20*(3), 863–876. https://doi.org/10.1177/09731296241228925.

Stienstra, R., Joosten, L. A. B., Koenen, T., van Tits, B., van Diepen, J. A., van den Berg, S. A. A., Rensen, P. C. N., Voshol, P. J., Fantuzzi, G., Hijmans, A., Kersten, S., Müller, M., van den Berg, W. B., van Rooijen, N., Wabitsch, M., Kullberg, B.-J., van der Meer, J. W. M., Kanneganti, T., Tack, C. J., & Netea, M. G. (2010). The inflammasome-mediated caspase-1 activation controls adipocyte differentiation and insulin sensitivity. *Cell Metabolism*, *12*(6), 593–605. https://doi.org/10.1016/j.cmet.2010.11.011

Tsuda, T., Horio, F., Uchida, K., Aoki, H., & Osawa, T. (2003). Dietary cyanidin 3-O-β-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. *The Journal of Nutrition*, *133*(7), 2125–2130. https://doi.org/10.1093/jn/133.7.2125

Vandanmagsar, B., Youm, Y.-H., Ravussin, A., Galgani, J. E., Stadler, K., Mynatt, R. L., Ravussin, E., Stephens, J. M., & Dixit, V. D. (2011). The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nature Medicine*, *17*(2), 179–188. https://doi.org/10.1038/nm.2279

Vatier, C., Kadiri, S., Muscat, A., Chapron, C., Capeau, J., & Antoine, B. (2012). Visceral and subcutaneous adipose tissue from lean women respond differently to lipopolysaccharide-induced alteration of inflammation and glyceroneogenesis. *Nutrition and Diabetes*, *2*(12), e51. https://doi.org/10.1038/nutd.2012.29