Korean Journal of Physiology and Pharmacology, Volume 28, Issue 2, 2024
ISSN:1226-4512
DOI:10.25463/kjpp.28.2.2024.2

Evaluation of Extracts Containing Anthocyanins for Their Ameliorative Effects in an Animal Model of Insulin Resistance and Type 2 Diabetes

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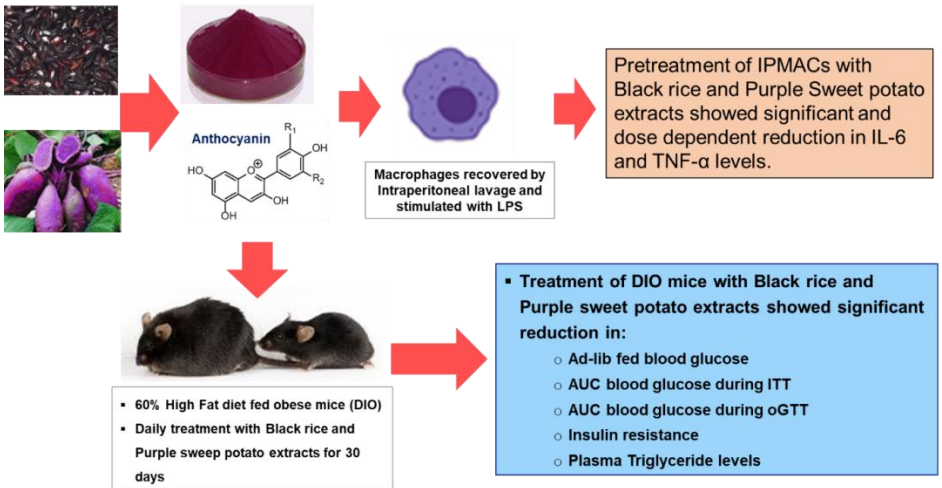
Abstract
Background: Nutraceuticals are the products which are being used as food and medicines for the treatment of several chronic diseases. Nutraceuticals are gaining much attention due to their easy availability, safety with no unwanted adverse effect, cheap and for their multiple therapeutic actions. Herbal 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 disorders associated with insulin signaling associated resistance and Diabetes.
Aim & Objectives: To investigate the effect of nutraceuticals for the treatment/improvement of inflammation associated conditions developed due to high calorie containing fatty diet and developed obesity, insulin signaling abnormalities and Type 2 Diabetes.
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 its effect on release of TNF-α and IL-6. Further, the effect of pretreatment with BR and PSP was evaluated on release of LPS induced TNF-α and IL-6 release in C57 BL/6 mice. In a separate experiment, Diet induced obese (DIO) mice (fed 60% high fat diet) were treated orally with extracts of BR and PSP at 100 mg/kg doses for 30 days to evaluate their effect on metabolic parameters and insulin resistance.
Results & Discussion: Extracts of black rice and purple sweet potato significantly reduced potent inflammatory cytokines like IL-6 and TNF-α in dose dependent manner in LPS challenged macrophages. Treatment of DIO mice with these extracts lowered fed blood glucose levels supporting the hypothesis that reducing inflammation may help improve insulin resistance. Extracts of BR and PSP also attenuated peripheral insulin resistance, as seen from significant improvement in insulin and glucose tolerance tests performed after multiple days of treatment.
Conclusions: The observed results demonstrate and support our hypothesis that extracts of black rice and purple sweet potato containing large amounts of Anthocyanins maybe useful agents in improving inflammation associated insulin resistance and Type 2 Diabetes.

Keywords: Anthocyanins; insulin resistance; cytokines; glucose intolerance; Type 2 Diabetes

Graphical Abstract:



I. INTRODUCTION

Metabolic disorders associated with insulin resistance (IR) and associated obesity, type 2 diabetes (T2D) and non-alcoholic fatty liver disease (NAFLD) are evident by a state of chronic inflammation developed due to over nutrition of fat [1]. Metabolic inflammation in obesity is different from that of Classical immune response to injury or infection and is modest and without apparent resolution over time [1]. It is well established that metabolic inflammation interferes with cellular metabolism and impairs insulin signaling in metabolically important tissues like liver, adipose, skeletal muscle, pancreas. The mechanisms by which these processes are triggered are still not defined completely [2].

Adipose tissue (AT) has been shown to play an important role for the inflammatory events in insulin resistance and associated obesity. Several cell types present in the adipose tissue contribute significantly to the inflammatory response during the development of insulin resistance and obesity. AT is known to play an important role in regulating fat mass and nutrient homeostasis. However, adipocytes also contribute to the chronic inflammatory response through secretion of various chemokines, cytokines and adipokines. These cells after their secretion lead to further events such as attracting macrophages into the adipocytes [3]. Once in the adipocytes, these macrophages get activated and act as a major trigger for release of pro-inflammatory cytokines and chemokines. This leads to continuous state of chronic inflammation which interferes with insulin signaling pathway thereby inducing insulin resistance and decreased sensitivity in insulin in metabolically important organs and cells [4]. This state of chronic nutrient overload and increased metabolic inflammation, adipocyte Hyperplasia and hypertrophy in conditions like obesity does not remain restricted to the AT. These secreted cytokines, chemokines also travel to other organs like liver skeletal muscle. These tissues further contribute significantly to the development of inflammation and interferes with insulin signaling pathways [5–7]. Further, resident macrophages in the liver also get activated and release proinflammatory cytokines leading to disruption of insulin signaling pathways and imparting insulin resistance [8]. This state of increased inflammatory condition in liver has been reported as increased expression of inflammatory genes following high-fat diet (HFD) feeding in mice with increase in insulin resistance and decrease in insulin sensitivity strongly supports the role of inflammation in this phenomenon [9,10].

Anthocyanins have been used since long as a nutraceutical, phytopharmaceutical, choleretic agent, appetite stimulant, and treatment for various chronic ailments. In recent years many potential health benefits are reported based on different cell based and animal studies. These health benefits are antioxidative effects, anti-inflammatory, prevention of cardiovascular diseases, as antidiabetic, anti-obesity, anticarcinogenic, improved visual health, antimicrobial, and neuroprotection (11).

It is reported that anthocyanins have mechanisms that can reduce insulin resistance, hyperglycaemia, inhibit gluconeogenesis, and actions of carbohydrate hydrolysing enzymes α-glucosidase and α-amylase and as such normalize glucose levels, improve insulin secretion and proliferation of pancreatic β-cells (12-14;). Cornus fruits, containing high levels of anthocyanins, are used in in Chinese prescription traditionally for the treatment of diabetes (15). The glycosides of cyanidin, delphinidin, and pelargonidin have been recognized as the primary bioactive components in Cornus kousa. Obese mice fed a diet high in cyanidin-3-glucoside from purple maize for 12 weeks lost weight and had lower white and brown adipose tissue weights. The study shows that when these obese rats were treated with purple corn diet, it showed several effects on metabolic parameters like reduction in glucose levels. There was also improvement in insulin, leptin and TNF-α mRNA levels near normalization (16). It has been shown that anthocyanins attenuate vascular and inflammatory responses to a high fat high energy meal challenge in overweight older adults and significantly lower IL-6 levels (17). Black current extracts (BCE) rich in anthocyanins treatment significantly reduced IL-1β and IL-6 mRNA levels post LPS challenge in RAW 264.7 macrophages. Also, pretreatment of RAW 264.7 macrophages with BCE attenuated mRNA abundance of IL-1β, IL-6 and TNF-α (18). Black Raspberry extract significantly reduced mRNA expression of pro-inflammatory genes in liver tissue such as nuclear factor-κB (NF-κB), interleukin (IL)-1β, IL-6, and cyclooxygenase-2 (COX-2) in rats fed High-Fat and High-Choline diets. Moreover, protein expression of NF-κB and COX-2 in liver tissue was also attenuated with Black Raspberry extract (19).

In view of above, objective of the present study was to(i) to understand the role of inflammation associated insulin resistance in diet-induced obesity (DIO) model of mice, and (ii) to determine whether extracts of black rice and purple sweet potato are effective in reduction/attenuation of high fat diet-induced obesity, inflammation, and insulin resistance in mice.

II. MATERIALS AND METHODS

2.1. Animals

Male C57BL/6J mice of 6 to 9 weeks of age were used in the experiment. All procedures followed the guidelines provided by the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) India. This study design and procedures followed herein have been approved by the Institutional Animal Ethics Committee (IAEC) of Eurofins Advinus Limited (Approval no: Renewal_008_April-2021). All the animals were maintained on a low-fat diet (LFD) consisting of 10% calories from fat (D12450B; Research Diets Inc., USA) before start of the experiment. Animals were then fed with a high-fat diet (HFD) consisting of 60% calories from fat (D12492; Research Diets Inc., USA) for the development of obesity (Diet induced obesity model).

2.2 Experimental designs and protocols:

2.2.1. Extraction of black rice and purple sweet potato:
Extraction of Black rice: 100 g of either black rice was cleaned by dry de-dusting using vacuum. The rice is coarsely powdered using grinder and powdered rice was transferred to 3L round-bottom flask. 500 ml of 80 % alcohol and 20 % water mixture was added and the flask was kept in water bath and the contents were refluxed for 3h. After 3h, the heating was stopped, and the contents were filtered. The resulting extract was concentrated under reduced pressure to about 100 ml. Then the concentrated extract was transferred to an evaporating dish and allowed to dry at ambient temperature. On drying it, the yield of extract was about 4 g dark purple colored pasty mass which was further dried to get a powder.
Extraction of Purple sweet potato: 100 g of skin was peeled and cleaned by dry de-dusting using vacuum. Peel was coarsely powdered using grinder and the powder was transferred to 3-liter round-bottom flask. 500 ml of 80 % alcohol and 20 % water mixture was added and the flask was kept in water bath and the contents were refluxed for 3h. After 3h, the heating was stopped, and the extract contents were filtered. The resulting extract was concentrated under reduced pressure to about 100 ml. Then the concentrated extract was transferred to an evaporating dish and allowed to dry at ambient temperature. On drying it, the yield of extract was about ~3.5 g dark purple colored pasty mass which was further dried to get a powder.

2.2.2. Proof of concept assay in lipopolysaccharide-primed peritoneal macrophages derived from C57BL/6 mice:
Phosphate buffered saline (PBS) was injected into the peritoneal cavity and after a few minutes mice were sacrificed by cervical dislocation. Then the peritoneal cavity was cut open and PBS in the cavity was aspirated. This aspirated PBS was centrifuged at 6000 RPM and macrophages were isolated. These macrophages were cultured in Dulbecco's minimum essential medium (DMEM) containing 10% fetal bovine serum (FBS) and 10 mg/mL gentamicin at 37°C in a humidified, 5% CO_2/95% air atmosphere. These macrophages were pretreated with different concentrations (0.3 to 6 mg/mL) of extracts of black rice and purple sweet potato and then stimulated with 1 ng/ml of lipopolysaccharide (LPS). Then the macrophages were incubated overnight, and the supernatants were collected. These supernatants were used for the estimation of inflammatory cytokines like TNF-α and IL-6 and using ELISA kits according to the manufacturer's manual for the respective kit for the procedure.

2.2.3. Acute effect of extracts on lipopolysaccharide-induced cytokine levels in C57BL/6 mice:
Male C57BL/6 mice of 8-10 weeks old were used for the experiment. These mice were treated with either Vehicle or various doses of BR and PSP extracts. Then 3 to 4 h post treatment, these mice were dosed by single intraperitoneal injection of LPS at 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
- Black rice extract, 30 mg/kg, PO + LPS, 1 mg/kg, IP
- Black rice extract, 100 mg/kg, PO + LPS, 1 mg/kg, I
- Black rice 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 TNF-α and IL-6 levels using commercially available ELISA kits according to the manufacturer's manual for estimation.

2.2.4. Chronic in vivo animal study
Male C57BL/6 mice of 6-9 weeks old were fed on LFD for control group (n=6) and with HFD for 12-16 weeks to develop diet induced obese mouse. Once the mice were obese post HFD feeding, ad-lib fed blood glucose levels were measured using glucometer and strips (AccuSure Simple®, MicroGene Diagnostic Systems Pvt Ltd). Animals were then randomized according to ad-lib fed blood glucose and body weight into different 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 on the basis of mg/kg of body weight. All the animals were monitored daily for clinical signs, general behavior and well-being. Body weight, Ad-lib fed blood glucose and food intake were monitored before dose and on day 7, 14, 21 and 28. On day 30, animals were fasted for 6h and blood samples were collected under anesthesia by retro-orbital puncture. Blood samples were centrifuged, and plasma samples were separated for the estimation of plasma triglycerides and total cholesterol levels. Also, white adipose tissues (WAT) and liver were collected and stored at -70^{0}C for further analysis.

2.2.5. Insulin and Glucose tolerance test for the assessment of insulin sensitivity:
Insulin tolerance test (ITT) and oral glucose tolerance test (oGTT) were performed on day 21 and 28, respectively. For ITT, 0.75 IU/kg of human insulin (Actrapid) was injected intraperitoneally in the fed state. For oral glucose tolerance test (oGTT), after 14h of fasting, glucose levels were monitored and then animals were orally dosed with 2g/kg of glucose [21]. For all the measurements, blood samples were collected by tail snip method. Blood glucose levels were measured using glucometer and strips (AccuSure Simple®, MicroGene Diagnostic Systems Pvt Ltd).) before the treatment with extracts (pretreatment) and 1h post treatment as baseline and at 0 min (pre-dose of insulin or glucose) and at 15

min, 30 min, 60 min, 120min and 180 min post dosing of insulin or glucose respectively. Area under curve (AUC) was calculated for glucose from 0 to 180 min.

2.2.6. Plasma measurements

After 30 days of treatment, plasma samples were collected and total cholesterol (TC), and triglycerides (TGs) concentrations were measured with commercially available diagnostic kits.

2.2.7. Statistical analysis

All the values in graphs are presented as mean ± SEM. All the parameters in the study were analyzed by applying one-way ANOVA and *post hoc* Dunnett's test. In case of $P < 0.05$, the difference was considered statistically significant.

III. RESULTS

3.1. Black rice and purple sweet potato extracts inhibit LPS-induced IL-6 and TNF-α levels in intraperitoneal macrophages (IPMACs):

In order to check if black rice and purple sweet potato extracts have inhibitory effect on inflammatory cytokines, we analyzed TNF-α and IL-6 levels by ELISA. Resident peritoneal macrophages were incubated with either vehicle or various concentration 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 significantly increased in LPS-primed intraperitoneal macrophages. Both BR and PSP extracts showed significant inhibition of IL-6 and TNF-α in LPS primed macrophages in a dose-dependent manner (Fig. 1A, 1B, 1C and 1D). This data suggests that BR and PSP extracts containing anthocyanins have a potential inhibitory effect on inflammatory cytokines.

3.2. Black rice and purple sweet potato extracts inhibit LPS-induced IL-6 and TNF-α levels in C57BL/5 mice:

To check the translation of observed effects in isolated IPMACs under in vitro conditions to in vivo conditions, black rice and purple sweet potato extracts were administered orally to C57BL/6 mice followed by LPS treatment. Both IL-6 and TNF-α levels were significantly increased in LPS treated mice as compared to saline treated mice. Pretreatment of these mice with BR and PST extracts significantly reduced plasma IL-6 and TNF-α levels as compared to LPS treated mice in a dose-dependent manner (Fig. 2A, 2B, 2C and 2D). This data suggests that BR and PSP extracts containing anthocyanins show inhibition of inflammatory cytokines release when stimulated by inflammatory mediators like LPS under in vivo conditions.

3.3. Effect of black rice and purple sweet potato extracts treatment on fed blood glucose, body weight, and feed intake in DIO mice:

To determine the effect 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 day 1, 7, 14 and 21 during the course of treatment. 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 as compared to control animals treated with vehicle. The reference standard treatment with Pioglitazone (30 mg/kg) also showed a significant improvement in fed blood glucose levels as compared to control animals treated with vehicle (Fig. 3). Body weight was increased linearly in mice fed the HFD as compared to LFD controls. Treatment of obese mice with BR and PSP extracts and Pioglitazone did not show significant effect on body weight and feed intake.

3.4 Effect of Black rice and purple sweet potato extracts on intraperitoneal insulin tolerance test (ITT) and oral glucose intolerance tests (oGTT) in DIO mice

Long-term feeding of high fat diet led to a significant increase in adipocyte weight, and accumulation of triglycerides, free fatty acids in adipocytes, liver and muscles, thereby increase in insulin resistance or decrease in insulin sensitivity [22, 23]. There is also chronic inflammation in these metabolically important tissues with significant increases and release of inflammatory cytokines like IL-6 and TNF-a levels in circulation. Therefore, in the present study we examined whether treatment of HFD fed mice with Black rice and purple sweet potato extracts reduces insulin resistance and thereby improves insulin sensitivity after chronic treatment. Interestingly, during insulin tolerance test, our results also show significant reduction 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 compared to vehicle treatment indicating significant improvement in insulin sensitivity during IIT. This indicates the insulin sensitizing effect of these extracts. Pioglitazone, a very well-known insulin sensitizer, showed a significant increase in insulin sensitivity in mice after chronic treatment as compared to vehicle control animals (Fig. 4).

BR and PSP extracts treated animals showed significant reductions in blood glucose levels during oral glucose-tolerance test (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 the blood glucose levels significantly in comparison with vehicle treatment group (Fig. 5A and 5B).

3.4 Effect of black rice and purple sweet potato extracts on adipose tissue-derived inflammatory cytokines

To investigate whether black rice and purple sweet potato extracts inhibit pro-inflammatory cytokines secreted in obesity, adipose tissues were collected at the end of the study and IL-6 and TNF-α levels were estimated using ELISA kits. Elevated levels of both IL-6 and TNF-α were

detected in the homogenized adipose tissue samples indicating the secretion of these cytokines by the adipose tissue in obesity. High fat diet-mediated obesity and associated insulin resistance lead to the production of inflammatory cytokines including IL-6 and TNF-α. In this study, BR and PSP extracts did not show statistically significant changes in any of these cytokines in the adipose tissue homogenates. Pioglitazone (30 mg/kg) showed significant reduction of IL-6 and TNF-α levels in the adipose tissue homogenates suggesting its anti-inflammatory effect in obesity-induced adipose tissue inflammation (Data not shown).

Although, black rice and purple sweet potato extracts showed significant and dose-dependent inhibition of TNF-α and IL-16 in LPS stimulated peritoneal macrophages, treatment at 100 mg/kg doses did not show significant inhibition of these cytokines in adipose tissues of DIO mice. This might be due to low dose of extracts administered and there might have been significant inhibition at its higher doses.

3.5. Effect on liver and adipose tissue weights

Feeding mice with a high fat diet is expected to increase tissue weight. In our study also, liver, and white adipose tissue weights were significantly increased in HFD fed mice as compared to the tissues from lean control mice fed with LFD. Pioglitazone is known for its disadvantage of weight gain after chronic treatment. In our study too, there was a significant increase in whole liver weight and white adipose tissue weight in animals treated with Pioglitazone. BR and PSP extract treatment had no significant effect on weight of liver and adipose tissue (data not shown here).

3.6. Effect on treatment on plasma triglycerides and total cholesterol levels in DIO mice

High fat diet fed obese mice exhibited significantly higher plasma levels of total cholesterol and triglycerides as compared to their lead control LFD fed C57BL/6J mice (Fig. 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 as compared to vehicle treated animals. Reduction in elevated total cholesterol in HFD fed mice was not affected by treatment with BR and PSP extracts as well as Pioglitazone treatment.

IV. DISCUSSION

Obesity and associated insulin resistance is ultimately the result of chronic, low-grade inflammation contributed by production of pro-inflammatory cytokines and infiltration of immune cells [1,30]. Elevated pro-inflammatory cytokines including TNF-α, IL-1β and IL-6 reduce insulin sensitivity through inhibition of insulin signaling [31-33] and plays crucial role in initiating the inflammatory response in obesity and associated insulin resistance. In view of this, we studied involvement of pro-inflammatory cytokines in diet-induced obesity and associated insulin resistance by studying the effect of chronic treatment with black rice and purple sweet potato extracts containing anthocyanins in 60% fat containing diet fed obese mice.

In vitro target engagement assays were performed to confirm whether 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 dose-dependent manner in LPS-stimulated intraperitoneal macrophages (IPMACs). This data suggests that BR and PSP extracts have a very significant anti-inflammatory activity which could be attributed to its mechanism of anti-inflammatory activity and reduction of associated secretion of pro-inflammatory cytokines [17]. Further, pretreatment of normal C57BL/6 mice with these extracts significantly reduced LPS induced cytokines production indicating the translation of effect from in vitro to in vivo condition and supporting our hypothesis that these extracts have anti-inflammatory properties.

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

Obese type 2 diabetic patients who show reduction in inflammation show improved glucose homeostasis due to attenuation by various extracts containing anthocyanins. [20]. Therefore, we also evaluated the effect of the extracts on *ad libitum* fed blood in DIO mice and found that there was significant improvement in ad-lib fed blood glucose from day 14, over the course of treatment in comparison to the vehicle treatment group.

To further understand the role of anthocyanins in regulating insulin and glucose homeostasis, we carried out ITT and oGTT in HFD fed obese. Despite HFD fed obesity, BR and PSP extracts treated mice showed moderate improvement in insulin sensitivity during IIT as seen from lowered blood glucose levels compared to vehicle treatment. Furthermore, blood glucose levels of BR and PSP extracts treated mice were significantly lower during oGTT. These findings support our hypothesis that reduction in chronic inflammation leads to improvement in insulin sensitivity, thereby improvement in blood glucose homeostasis.

Observed beneficial effect of BR and PSP extracts on insulin sensitivity and glucose homeostasis in DIO mice were not well correlated with

feed intake and body weight as both the treatments did not show significant effect on body weight and feed intake.

IL-6 and TNF-α being major contributing pro-inflammatory cytokines to the obesity-mediated inflammation and resultant insulin resistance, pancreatic β-cell dysfunction and pathogenesis of type 2 diabetes [30,35], we evaluated the effect of these extracts on these cytokines in vitro as well as in vivo. Our study in adipose tissue homogenates confirms that pro-inflammatory cytokines including IL-6 and TNF-α were released in obesity in significant quantities. In the present study, BR and PSP extracts attenuated IL-6 and TNF-α levels in LPS challenged peritoneal macrophages, however, it failed to significantly inhibit these cytokines in adipose tissue homogenates. This may be due to shorter duration of treatment in present study, and it might inhibit these pro-inflammatory cytokines at longer duration treatment with higher doses which is not tested in the present study.

Production of cytokines is implicated in the regulation of lipid metabolism and glucose homeostasis [21, 36]. More precisely, secretion of IL-6 affects lipid metabolism, thereby induction of hypertriglyceridemia through inhibition of lipoprotein lipase activity [37-39]. In view of this, we examined the effect of BR and PSP extracts on plasma triglyceride and total cholesterol levels in DIO mice. High fat diet fed obese mice exhibited significantly higher plasma levels of total cholesterol and triglycerides compared to their lean control (40). Following 30 days 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 VLDL or by increased clearance out of plasma. This shows that reduction in chronic inflammation also decreases DIO-associated high TG, an independent cardiovascular risk factor and the barometer of metabolic health. However, no significant difference in total cholesterol level was observed.

To conclude, our data reveals that BR and PSP extracts, inhibitors of inflammation reduce obesity and associated insulin resistance in mouse model of high fat diet-induced obesity. BR and PSP extracts significantly lowered fed glucose levels, improved insulin resistance and glucose intolerance. BR and PSP extracts were capable of significantly inhibiting the release of pro-inflammatory cytokines in LPS-induced peritoneal macrophages as well as in C57BL/6 mice. Collectively our findings support the hypothesis that long term treatment with anthocyanin rich extracts of BR and PSP could be useful agents in improving inflammation associated insulin resistance and type 2 diabetes.

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Table 1. Different animal groups and treatments administered in chronic in vivo animal study.

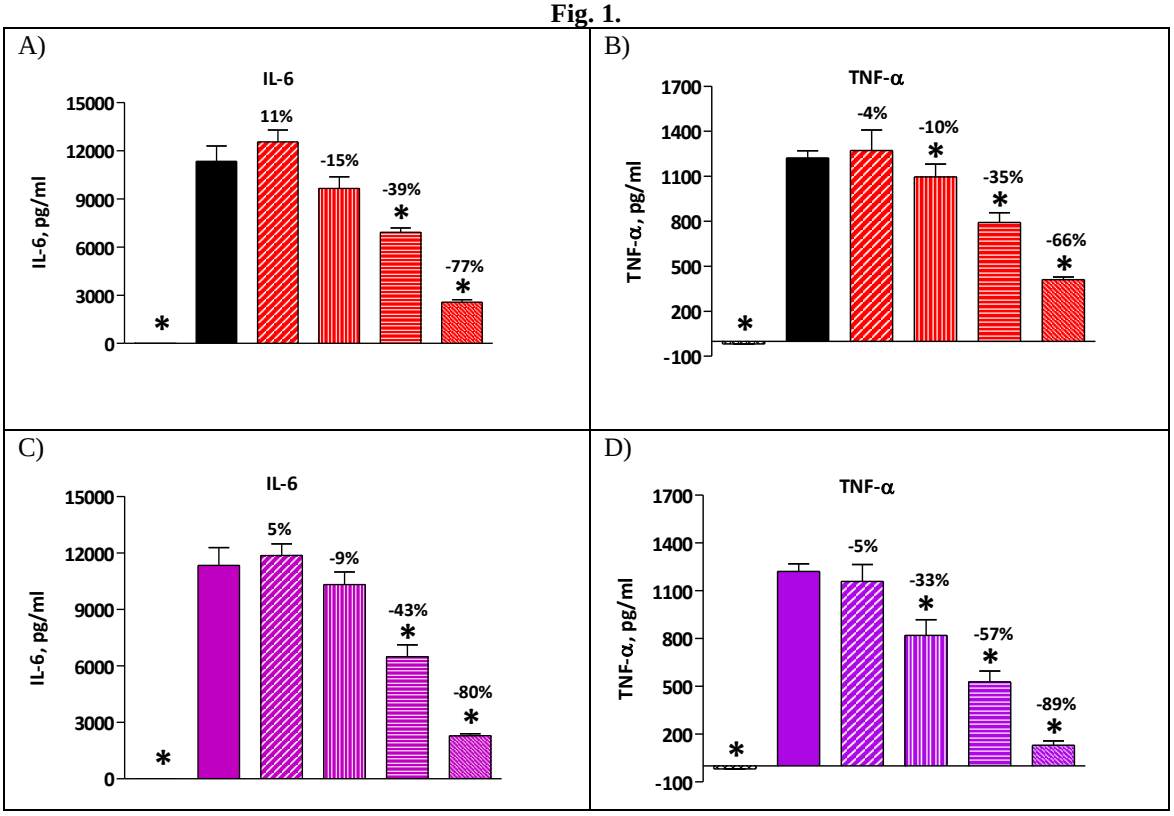
Study groups (n=6)	Diet Fed	Treatment Received

 DOI:10.25463/kjpp.28.2.2024.2

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.

Figure Legends

Fig. 1.



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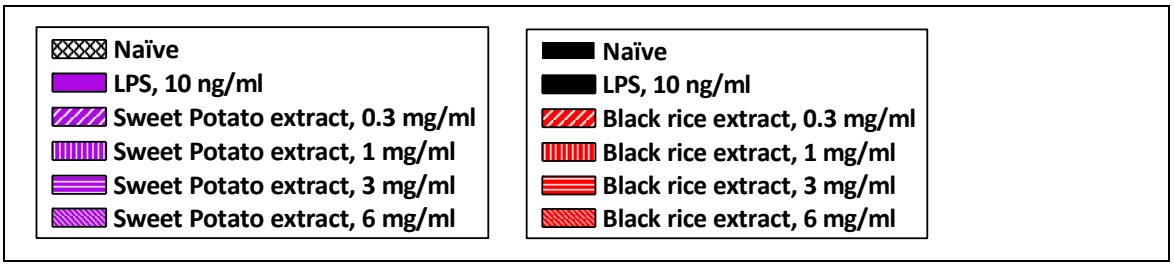

Fig. 1. Effect of black rice and purple sweet potato extracts (3-300 mg/mL) on (A) IL-6 and (B) TNF-α levels in LPS-stimulated mouse peritoneal macrophages. **P*<0.05 compared to LPS. Black rice and purple sweet potato extracts showed significant and dose dependent reduction in TNF-α and IL-6 levels as compared to vehicle treatment.

Fig. 2.

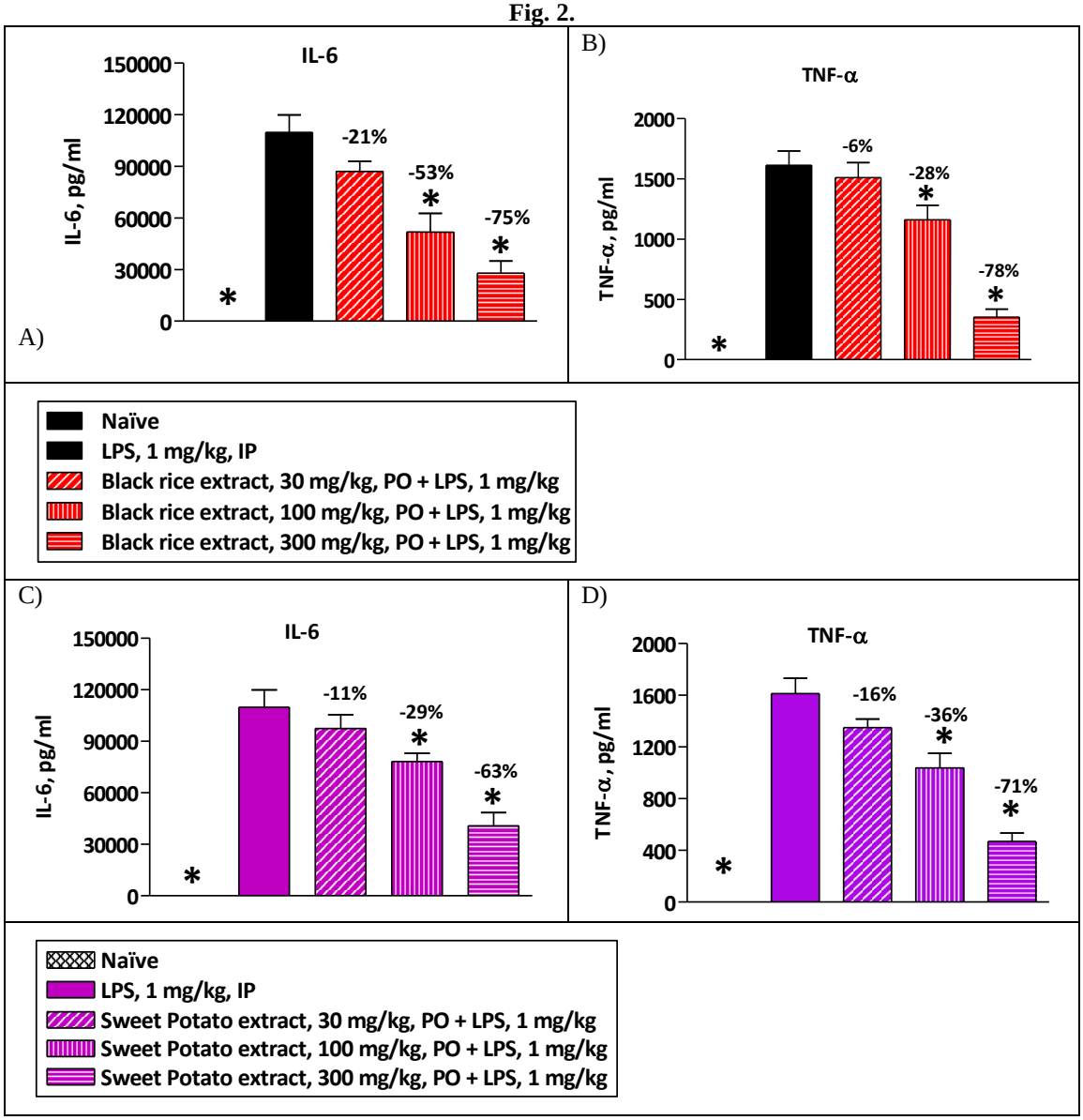

Fig. 2. The effect of black rice and purple sweet potato extracts (30-300 mg/kg) on LPS induced changes in plasma levels of (A) IL-6 and (B) TNF-α levels in C57BL/6 mice. Data is shown as mean ± SEM **P*<0.05 compared to LPS treated mice. Black rice and purple sweet potato extracts showed significant and dose dependent reduction in plasma levels of IL-6 and TNF-α levels as compared to LPS challenged treated mice.

DOI:10.25463/kjpp.28.2.2024.2

Fig. 3.

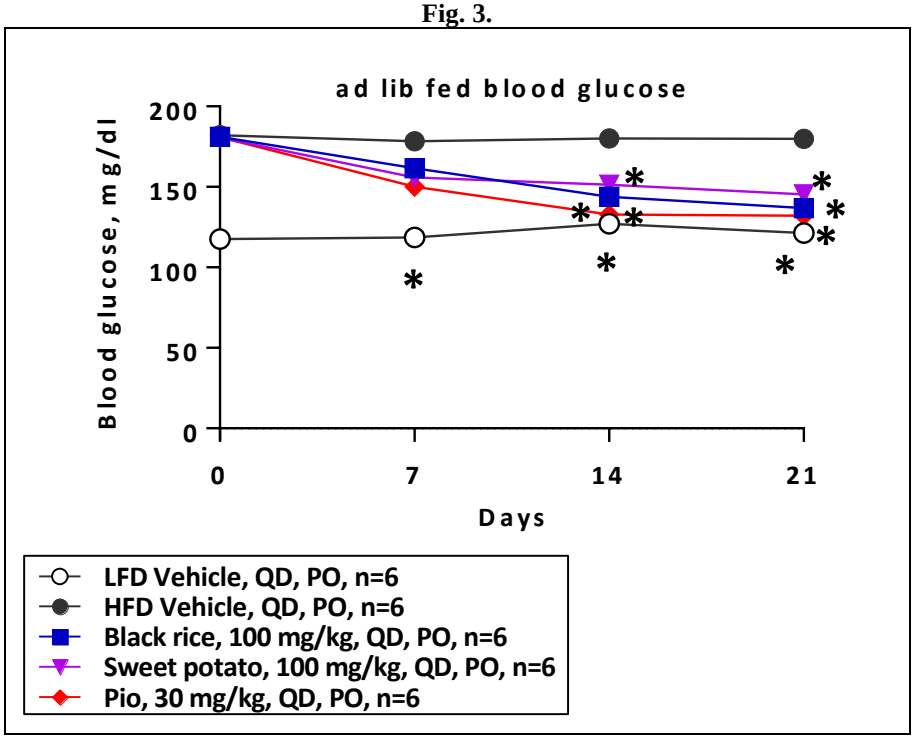

Fig. 3. Effect of black rice and purple sweet potato extracts (100 mg/kg) and Pioglitazone (30 mg/kg) on ad libitum fed blood glucose (n=6). Data is shown as mean ± SEM. *P<0.05 compared to vehicle treatment. Black rice and purple sweet potato extracts and Pioglitazone showed significant reduction in ad-lib fed blood glucose as compared to vehicle treatment on day 14 and day 21.

Fig. 4.

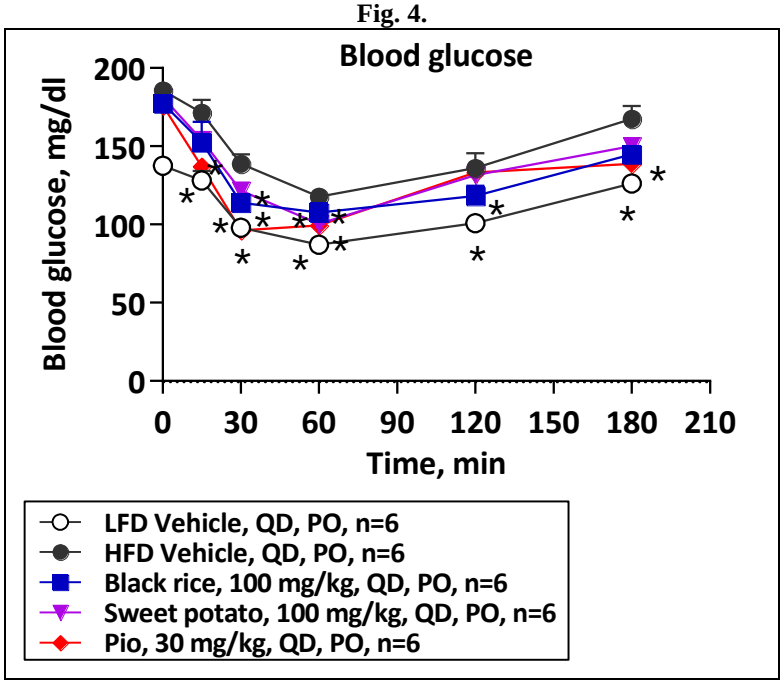

Fig. 4. Effect of Effect of black rice and purple sweet potato extracts (100 mg/kg) and Pioglitazone (30 mg/kg) treatment on insulin sensitivity. Data is shown as mean ± SEM *P <0.05 compared to vehicle treatment, One-way ANOVA followed by Dunnett's multiple comparison test,

 DOI:10.25463/kjpp.28.2.2024.2

*P<0.05 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.

Fig. 5.

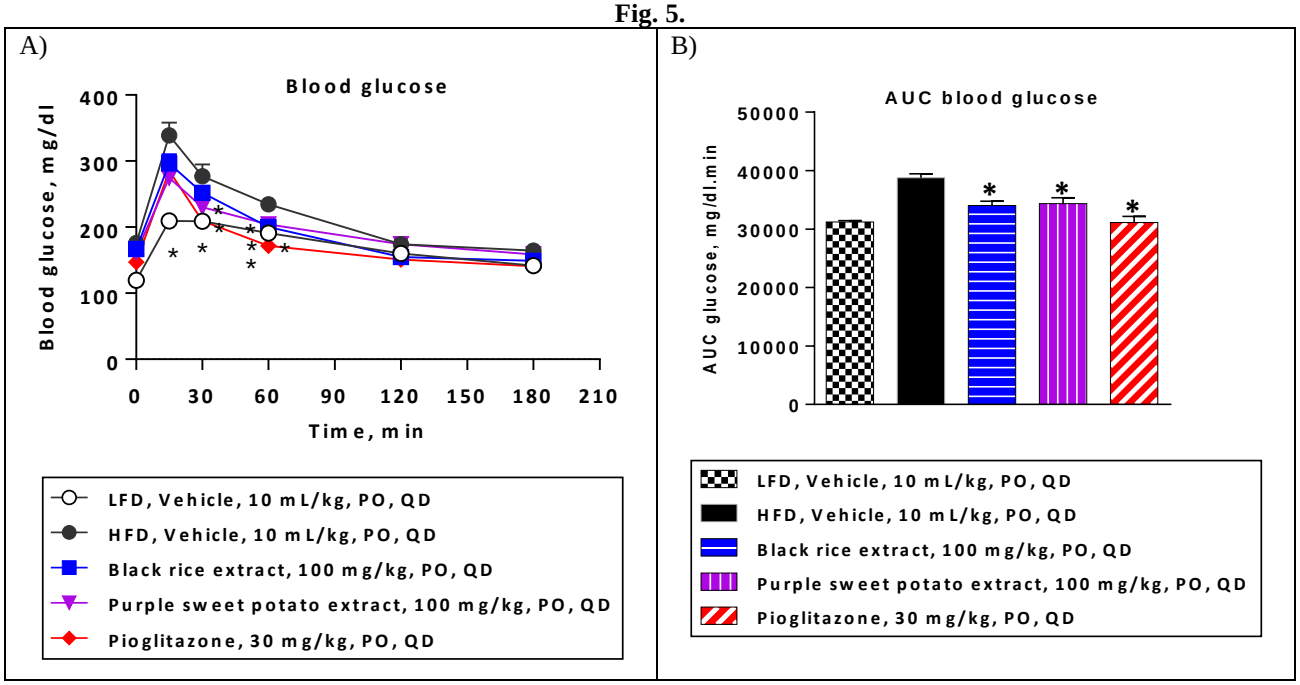

Fig. 5. Effect of Effect of black rice and purple sweet potato extracts (100 mg/kg) and Pioglitazone (30 mg/kg) treatments on glucose tolerance. Data is shown as mean ± SEM *P<0.05 compared to vehicle, One way ANOVA followed by Dunnett's multiple comparison test. Both black rice and purple sweet potato 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.

Fig. 6.

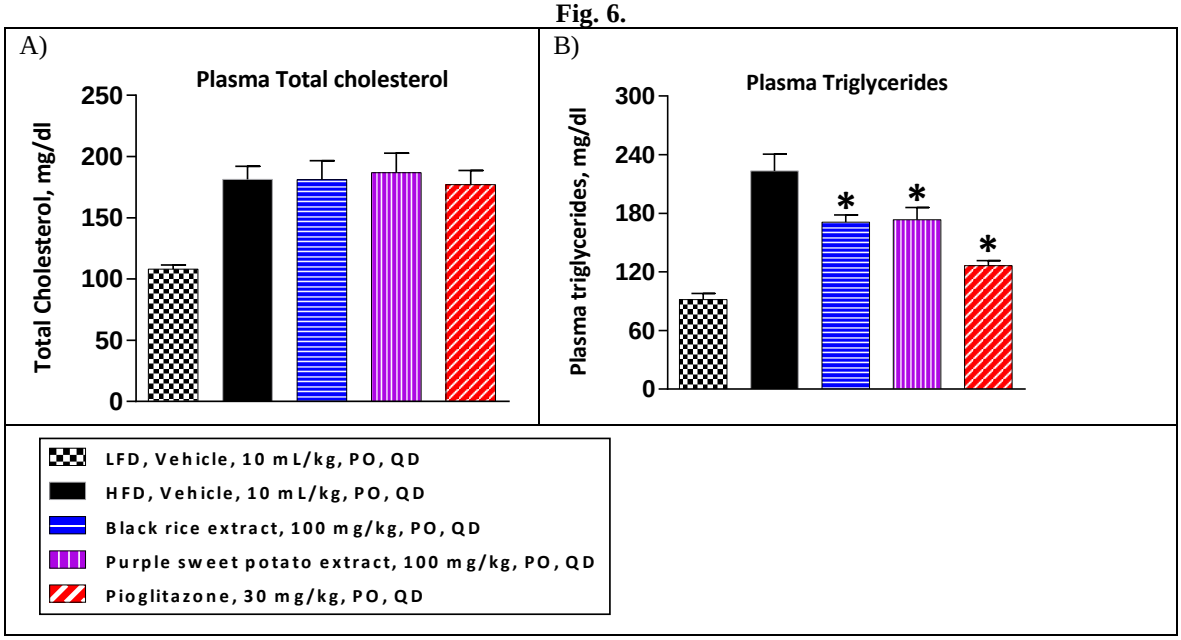

Fig. 6. Effect of black rice and purple sweet potato extracts (100 mg/kg) and Pioglitazone (30 mg/kg) treatments on plasma (A) total cholesterol (TC) and (B) triglycerides (TG) levels. Data is shown as mean ± SEM *P<0.05 compared to vehicle treatment. One way ANOVA followed by Dunnett's multiple comparison 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.