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Research Article

Anti-Diabetic Activity and Metabolic Changes in Purple Rice Bran Supplement Type 2 Diabetic Rats by Proteomics

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ABSTRACT
Purple rice (*Oryza sativa* L. *indica*) has been shown to have anti-hyperglycemic activity. However, the underlying mechanism of the anti-hyperglycemic activity of purple rice is still unknown. To explore this mechanism, we studied the protein profiling of the liver tissues of normal rats, normal rats with purple rice bran supplementation, type 2 diabetic rats, type 2 diabetic rats with purple rice bran supplementation, and type 2 diabetic rats with metformin treatment by using one-dimensional gel electrophoresis and quantitative LC-MS/MS analysis. Eight proteins and five proteins were solely expressed in type 2 diabetic rats and type 2 diabetic rats with purple rice bran supplementation respectively. The unique proteins in the diabetic rats correlated with the fatty acid synthesis, supporting the view that abnormal lipid metabolism and hyperlipidemia are complications of diabetes. The unique proteins in the diabetic rats with purple rice bran supplementation correlated with the insulin signaling pathway, suggesting that purple rice bran might improve insulin sensitivity. Interestingly, 11 proteins co-expressed in both normal control rats and type 2 diabetic rats with purple rice bran supplementation involved in oxidative stress response and autophagy. Our study might help to unveil the molecular mechanism involved in the anti-diabetic activity of purple rice.

Keywords: liver, purple rice bran, proteomics, rat, type 2 diabetes.

INTRODUCTION

Type 2 diabetes mellitus is a common chronic metabolic disease which is characterized by hyperglycemia. Diabetes results from impaired pancreatic β cell functioning which leads to failure to secrete adequate insulin or insulin resistance, or both[1]. The proposed mechanisms that explain pathogenesis of diabetes are endoplasmic reticulum stress; oxidative stress; and ectopic lipid deposition in the muscle, liver, and pancreas[2]. The liver is a major organ for the regulation of glucose homeostasis, fatty acid metabolism, and lipoprotein metabolism. Hepatic insulin resistance is a major contributing feature of type 2 diabetes[3,4].

In the past, several types of type 2 diabetes drugs such as oral anti-diabetic agents (OAAs), insulin, and incretin-based drugs have been developed for controlling blood glucose via different pathways[5]. However, current anti-diabetes drugs exhibit fewer efficacies and have undesirable side effects. In addition to these, current anti-diabetes drugs seem to be able to just relieve the blood glucose level and not cure type 2 diabetes[6].

Medicinal herbs play an important role in treating human diseases. Over 400 plants have been evaluated for type 2 diabetes treatment[7]. In our study, we focused on purple rice (*Oryza sativa* L. *indica*) which is cultivated in China and South-East Asia, including Thailand. It has been consumed as a traditional food in China and eastern Asian countries for many years[8]. Purple rice has a high content of anthocyanins, which are water soluble pigments. They are widely available in human diet, in cereals, beans, fruits, vegetables, and red wine.

Anthocyanin has been proved to have antioxidant, anti-inflammatory, anti-hyperglycemic, and anti-hyperlipidemia effect. A study in purple rice extract treated male Sprague-Dawley rats reported the effects of anthocyanin as including reducing of blood glucose, increasing of insulin secretion, protection from free radical induced damage, and improving of insulin resistance[9]. Rice bran oil improves lipoprotein metabolism via up-regulation of LDL receptors in the liver and disposal of cholesterol and biliary salts in the guts in streptozotocin-induced diabetic rats[10]. Recently, it was proposed that purple rice extract inhibits α-glucosidase, α-amylase, and aldose reductase activities in diabetic rat models. In a small-scale human study, low dose of purple rice extract was found to effectively suppress postprandial increase in blood glucose levels[11]. However, the molecular mechanism underlying the anti-diabetes action of purple

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rice remains unknown and needs further in-depth studies. In the present study, we used proteomic to explore the molecular mechanism of purple rice in hypoglycemia and hyperlipidemia.

MATERIALS AND METHODS

Induction of HFD-fed and STZ-induced type 2 diabetic rats

The protocol of this experiment was approved by Animal Ethics committee, the Faculty of Medicine, Chiang Mai University, Thailand (protocol number- 27/2558). The National Laboratory Animal Center, Mahidol University, supported 180–200gram body weight of adult Male Wistar rats. The rats were adopted under controlled temperature at 25±2℃ with a 12-hour light: dark cycle. The rats were allocated two dietary regiments: normal diet (ND) (20% fat of total energy) and high-fat diet (HFD) (60% fat of total energy) ad-lib for the initial period of 2 weeks. After the initial period, the HFD-fed rats were injected with streptozotocin (STZ) intraperitoneally at a dose of 35 mg/kg body weight; meanwhile, the control group was injected with citrate buffer. The fasting blood glucose levels were measured at the 2nd week after injection of the citrate buffer or STZ. In this study, the rats whose fasting blood glucose levels were ≥250 mg/dl were regarded as type 2 diabetic rats.

Experimental design

In this study, a total of 25 rats were used, and they were divided into five groups consisting of five rats per group. Group I: normal rats (NC); Group II: normal rats that received purple rice bran at 50 g/kg diet (NC-PR); Group III: type 2 diabetic rats control (DM2C); Group IV: DM2 rats that received high-fat diet with purple rice bran supplement 50 g/kg diet (DM2-PR); Group V: DM2 rats that received the reference drug (metformin) at a dose of 30 mg/kg BW (DM-Drug). Metformin was administered once daily by gavage feeding for 8 weeks. The rats were then sacrificed, and blood and the liver tissues were collected for further biochemical analysis.

Biochemical analysis

At the 8th week of administering the purple rice bran supplement, fasting plasma samples were collected from the rats. Fasting plasma glucose, cholesterol, and triglyceride (TG) levels were determined by using commercial enzymatic colorimetric kits (Biotech, Bangkok, Thailand). Meanwhile, the plasma-free fatty acid level (non-esterified fatty acid) was determined by using an enzymatic ready-make kit from Japan (NEFA C, Wako Pure Chemical). For the HbA1c concentration analysis, the samples were sent to Central Laboratory, Maharaj Nakorn Chiang Mai, on the same day of sample collection, and HbA1c concentration was measured by High Performance Liquid Chromatography (HPLC). Rat/Mouse Insulin ELISA Kit from LINCO Research, USA, was used to analyze the fasting plasma insulin level, and this is the Sandwich ELISA method.

Homeostasis model assessment of insulin resistance (HOMA-IR) and homeostasis model assessment of β cell function (HOMA-β)

HOMA-IR was used to calculate the insulin resistance. The formula for HORA-IR is presented below[12].

$$\text{HOMA-IR} = \frac{\text{fasting insulin level (μU/ml /dl)} \times \text{fasting glucose level (mg/dl)}}{405.1}$$

For calculating the pancreatic β cell function, HOMA-β was used. The following formula was used to calculate HOMA-β. [13]

$$\text{HOMA-}\beta = \frac{\text{Fasting insulin level (U/l)} \times 20}{\text{Fasting glucose level (mmol/l)} - 3.5}$$

Tissue homogenization and protein concentration determination

Frozen liver samples (100mg per rat liver) were pulverized in liquid nitrogen using a mortar and pestle. Then 0.5% SDS was added to the powdered samples and transferred into new eppendorf tubes. The proteins were purified by the acetone precipitation method. Finally, the protein pellets were resuspended in 0.5% SDS and stored at −20℃. The protein concentration was determined by Lowry's method using bovine serum albumin (BSA) as the standard. Pooled samples of the different groups were made by mixing equal amounts of protein (40 μg) from individual tissue samples.

Determination of protein expression by SDS-PAGE analysis

The proteins from the different protein samples were separated by SDS-PAGE gel electrophoresis (12.5% separating gel and 4% stacking gel). After completion of electrophoresis, the protein bands were visualized by silver staining. The silver stained gels were scanned by a GS-710 calibrated imaging densitometer. The gel images were exported and analyzed by an image analysis software program (Quantity one version 42.0). The protein bands were cut manually into small pieces of about 1 mm^3 using scalpel and forceps. The gel pieces were placed in a 96-well microtiter plate (~8–10 pieces per well). The gel pieces were soaked in 100 μl of sterile water and stored at 4℃ until further investigation.

Analysis of protein by mass spectrophotometry and data analysis

The gel pieces were in-gel digested using an in-house protocol developed in the Proteomics Research Laboratory, Genome Institute, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Thailand. For protein quantitation, DeCyder MS 2.0 Differential Analysis software (GE Healthcare, Sweden) was used. The acquired MS/MS raw data were converted to mzXML files using CompassXport 1.3.10 (Bruker Daltronics, Germany) prior to importing into the DeCyder MS software. The data from the DeCyder MS software were put into the Mascot software version 2.2 (Matrix Science, UK) for database search. The data were searched against the NCBI database for protein identification. The group of proteins of interest obtained from the Mascot search was categorized based on the biological process. Uniprot was used to search for the molecular weight and the understanding of the molecular function and the biological processes. STITCH (v.4.0) was used to find out whether the proteins are insulin dependent or not. STRING (v.10) was used to predict the protein–protein interaction

Table 1: Effects of Purple Rice Bran Supplement for 8 Weeks on Metabolic Variables in Type 2 Diabetic Rats.

	NC	NC-PR	DM2C	DM2-PR	DM-drug
Body weight, g	432.00±1.22	442.00±8.74	500.62±8.15*	513.45±11.19	485.62±14.18
Visceral fat mass, g	28.11±1.42	36.48±5.04	50.54±3.98*	50.74±4.01	47.00±3.69
Energy intake, kcal/day	87.07±1.34	105.40±1.63*	129.69±7.64*	122.26±2.11	123.21±2.03
Fasting Plasma Parameters					
Glucose, mg/dl	154.45±6.18	151.50±5.85	290.73±5.82*	214.86±7.82¥	235.34±5.09¥
HbA1c, mg/dl	3.75±0.05	3.70±0.00	6.07±0.57*	4.07±0.19¥	4.13±0.07¥
Insulin, ng/ml	2.84±0.23	3.37±0.47	4.24±0.14*	3.69±0.40	3.67±0.34
FFA, mmol/l	0.53±0.13	0.50±0.13	1.03±0.26*	0.67±0.06¥	0.63±0.06¥
TG, mg/dl	62.37±9.17	40.11±4.56*	113.77±3.14*	89.83±3.73¥	79.90±5.05¥
Cholesterol, mg/dl	85.92±5.28	85.69±4.43	118.99±9.72*	104.36±6.47	94.46±7.99¥
HOMA-IR (insulin resistance)	0.96±0.04	1.11±0.32	2.42±0.09*	1.68±0.28¥	1.12±0.13¥,§
HOMA-β (β cell function)	196.58±43.00	208.89±32.54	133.64±30.20	186.77±17.43	119.18±11.04

Notes: The mean±SD value was calculated from 5 rats: normal control (NC), normal control treated with purple rice bran (NC-PR), type 2 diabetic rats control (DM2C), type 2 diabetic rats treated with purple rice bran (DM2-PR), and type 2 diabetic rats treated with metformin (30 mg/kg body weight).
*p<0.05 vs NC, ¥p<0.05 vs DM2C, and §p<0.05 vs DM2-PR.

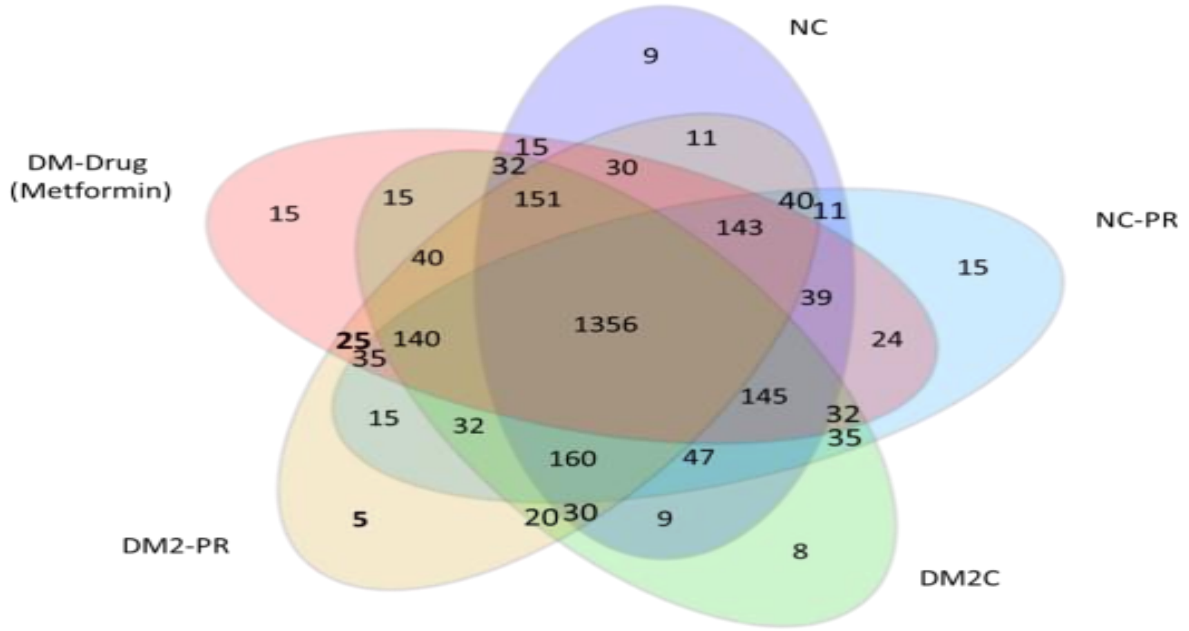

Figure 1: The Venn diagram of the distribution of the 2684 identified proteins in NC, NC-PR, DM2C, DM2-PR, and DM-Drug (metformin) rat livers.

network.

Statistical Analysis

Results are shown as mean ± SD. Statistical differences between the experimental groups were assessed using analysis of variance (ANOVA) confirmed by post hoc Fisher's test. For all analysis, a level of $p<0.05$ was considered as statistically difference.

RESULTS

The mean ± SD value of body weight (g), the visceral fat mass (g), and the biochemical parameters are summarized in Table 1. Upon doing a comparison between NC and NC-PR, it was observed that there were no significant changes in any of the parameters except energy intake, which increased in NC-PR. At the same time, the DM2C rats were found to have significantly higher values in all the parameters except in HOMA-β than NC, which indicates greater β cell dysfunction and insulin resistance. In the DM2-PR and the DM-drug groups, fasting glucose, HbA1c, FFA, and TG were found to be significantly lower when compared with the DM2C group. Additionally, it was observed that purple rice bran and drug treatment reduce fasting insulin, but not in a statistically significant manner. HOMA-IR was significantly lower in DM-drug group than DM-PR group. Interestingly, DM2-PR had higher HOMA-β than DM-drug, but statistically not significant**.**

LC-MS/MS analysis and protein identification

The tandem mass spectra data were searched against the NCBI database for protein identification. The search parameter was Rattus norvegicus as taxonomy. Overall, 2684 differentially expressed proteins were identified. The

Table 2: Protein Identification and Functional Classification of Proteins in and Diabetic Rats (DM2C).

Accession No.	Gene name	Protein name	Functional category	MW (kDa)	Peptide sequence
gi 113130	Chrm4	ACM4_RAT RecName: Full=Muscarinic acetylcholine receptor M4	cell signaling	52.9	MXNFTPVNGSSANQSVR
gi 149029666	Pa2g4	proliferation-associated 2G4, isoform CRA_b	cell signaling	35.46	MGGDIANR
gi 157818637	Slamf1	signaling lymphocytic activation molecule precursor	cell signaling	38.4	ILGNRR
gi 112984402	Spag5	sperm-associated antigen 5	cellular development	132.4	DAAIEEK
gi 110295237	Gulp1	CED-6	lipid metabolism	34	ILEPKTK
gi 158428968	Ctrb1	C Chain C, crystal structure of a trypsin-like mutant (S189d, A226g) chymotrypsin	protein metabolism	27.9	LQQAALPIVSEADCKK
gi 293349643	-	PREDICTED: ribosomal protein L7-like 1	translation	29.5	KLFSGVFVK
gi 149054380	-	similar to RIKEN cDNA 3010027G13, isoform CRA_a	unknown	31.1	SPGIR

Table 3: Protein Identification and Functional Classification of Proteins Diabetic Rats with Purple Rice Bran Supplement (DM2-PR).

Accession No.	Gene name	Protein name	Functional category	MW (kDa)	Peptide sequence
gi 13929126	Galnt5	polypeptide N-acetylgalactosaminyltransferase 5	catalytic enzyme	105.1	GMRPPRNGAGGK
gi 221325616	Camsap3	calmodulin-regulated spectrin-associated protein 3	cellular structure	136.7	HPLLSPGGPQSPLRGSTGSLK
gi 81910780	Klc3	KLC3_RAT RecName: Full=Kinesin light chain 3	motor protein	55.7	LRGESMAGAAGMK
gi 149028151	Khsrp	KH-type splicing regulatory protein, isoform CRA_b	translation	74.2	SGEMIKK
gi 197386987	-	uncharacterized protein LOC317617	unknown	86.5	MLAAMAAFLVK

numbers of proteins identified in the five groups are summarized in Figure 1. The total numbers of the identified proteins in NC, DM2C, and DM2-PR were 2228, 2252, and 2233, respectively. As depicted in Figure 1, the NC, DM2C, and DM2-PR had 9, 8, and 5 unique proteins, respectively. A total of 1356 proteins, representing ~50.5% of the total number of proteins identified, were found in all of the five groups.

The total number of proteins identified was NC = 2228 (9 unique proteins), DM2C = 2252 (8 unique proteins), and DM2-PR = 2233 (5 unique proteins). The 1356 overlapping proteins were common in all the five groups while 11 proteins shared between the NC and the DM2-PR groups. The number of proteins was found to be normal in the normal group but to have reduced in the DM2Cgroup; however, these proteins were rediscovered in the purple rice bran treated diabetic rats.

Protein identification and functional categories of unique and shared proteins found in NC, DM2C, and DM2-PR rat livers

To identify biomarkers for diabetes, we focused on the eight unique proteins in the DM2C group. To find out the molecular mechanism of the anti-diabetic effects of purple rice, we focused on the unique proteins in the DM2-PR group and the 11 proteins found in both the NC and the DM2-PR groups. The proteins of interest were investigated for belonging to the functional category against the UniProt database.

Seven out of the eight unique DM2C proteins exhibited functions such as cell signaling, cellular process, lipid metabolism, transcription, and protein metabolism (shown in Table 2). Four out of the five unique DM2-PR proteins were classified into catalytic enzymes, proteins based on cellular structure, motor proteins, and translation-related proteins (shown in Table 3). The 11 shared NC and DM2-PR proteins were categorized into autophagy-related proteins, catalytic enzymes, developmental proteins, hypothetical proteins, oxidoreductase proteins, and proteins modification (shown in Table 4).

Table 4: Identification and Functional Classification of Co-expressed Proteins in Control and Diabetic Rats with Purple Rice Bran Supplement.

Accession No.	Gene name	Protein name	Functional category	MW (kDa)	Peptide sequence
gi 16758562	Becn1	beclin-1	autophagy	51.6	LDTSFK
gi 158186687	Qtrt1	queuine tRNA-ribosyltransferase	catalytic enzyme	44.2	VEEAMHRSVR
gi 50811825	Prm3	protamine-3	developmental protein	11.5	GHESSMK
gi 6981568	Sod3	extracellular superoxide dismutase [Cu-Zn] precursor	oxidoreductase	26.6	QREADAR
gi 40018582	Gyltl1b	glycosyltransferase-like protein LARGE2	protein modification	79.3	YLTALQQSRSR
gi 157820067	RGD1309036	uncharacterized protein LOC292874	hypothetical protein	18.3	VPTCAPR
gi 149033718	-	enamelin (predicted), isoform CRA_b	unknown	136.1	APKIK
gi 149057425	-	rCG64374	unknown	15.1	MQIQNASI
gi 149063902	-	rCG23583	unknown	76.4	TNTPAR
gi 149061061	-	similar to F23N19.9 (predicted), isoform CRA_b	unknown	28.6	DVVYDIASQAHLHLK
gi 149039821	-	rCG24198, isoform CRA_b	unknown	23.2	MGVGGCPMAASYPQLSR

Bioinformatics analysis of unique and shared proteins found in NC, DM2C, and DM2-PR

The STITCH (v.4.0) chemical–protein analysis showed that the unique proteins were linked with metformin and not with insulin, revealing that unique proteins are related with type 2 diabetes mellitus [Figure 2(a)]. To get more information regarding the molecular mechanism of diabetes, we constructed the protein interaction network between the unique proteins of the DM2C group and other proteins by using the STRING (v.10) software. The result was as shown in Figure 2(b). Seven additional interacting proteins (Rpl7a, Egfr, Ptpn6, ApoB, ApoE, Ppara, and Agt) were added to get a more comprehensive figure of interaction. Interestingly, four out of six unique proteins (Pa2g4, Slamf1, Gulp1, and Chrm4) were found to be associated with proteins involved in the synthesis of apolipoproteins (ApoB and ApoE). However, Ctrb1 and Spag5 were not observed to have any interaction with these proteins.

The STITCH chemical proteins interaction software (v.4.0) confirmed that the unique proteins of the DM2PR rats are connected with metformin and not linked with insulin, pointing out those unique proteins are associated with non-insulin-dependent type 2 diabetes [Figure 3(a)]. The protein interaction network of unique protein in DM2PR rat livers was analyzed by using the STRING database. Ten additional interacting proteins (Galnt1, Muc1, Hsp90aa1, Egfr, Akt2, Akt1, Foxo3, Foxo1, Irs1, and Ctnnd1) were added to explore the possible mechanism of the anti-diabetic effect of purple rice [Figure 3(b)]. Interestingly, the unique proteins of the DM2-PR rat livers (Galnt5, Klc3, Khsrp, and Camsap3) showed a correlation with the proteins involved in insulin signaling (Akt1, Akt2, Foxo1, and Irs1).

After that, the 11 shared proteins found in NC and DM2-PR were studied to explore the effect of purple rice on rat liver. From the UniProt database, 5 out of 11 shared proteins (Becn1, Qtrt1, Prm3, Sod3, and Gyltl1b) were found their functions. One protein was entered in the list of UniProt, but its function is still under review. By using the STITCH chemical–protein interactions, it was established that the proteins are associated with metformin and not with insulin, meaning the proteins are of insulin-independent type 2 diabetes [Figure 4(a)]. Among them, only two proteins (Becn1 and Sod3) were found to have interaction between each other. Ten additional proteins (Atox1, Sod2, Bcl2l1, Bcl2, Park2, Uvrag, Pik3c3, RGD1305422, Atg14, and Pik3r4) were subjected to STRING analysis for revealing a more complete map of the interaction [Figure 4(b)]. As seen in Figure 4, these proteins are involved in autophagy (Atg14, Pik3r4, and Pik3c3) and antioxidant pathways (SOD2). Autophagy and antioxidant pathways are cell rescue mechanisms, and so, purple rice may contribute to cell survival mechanism in liver tissues.

DISCUSSION

In our study, the biochemical parameters of purple rice bran supplemented normal rats showed no significant changes from those of normal rats, excluding energy intake and fasting TG. This means that purple rice may not harmful to normal rats. In addition, purple rice bran supplement can help to lower the TG level in a healthy person[14]. Type 2 diabetes is caused by insulin resistance, not due to pancreatic β cell mass or functions. In our study, changes in HOMA-β did not reach statistically significant level, meaning that disturbance in the pancreatic β cell mass or function was not obvious. A combination of

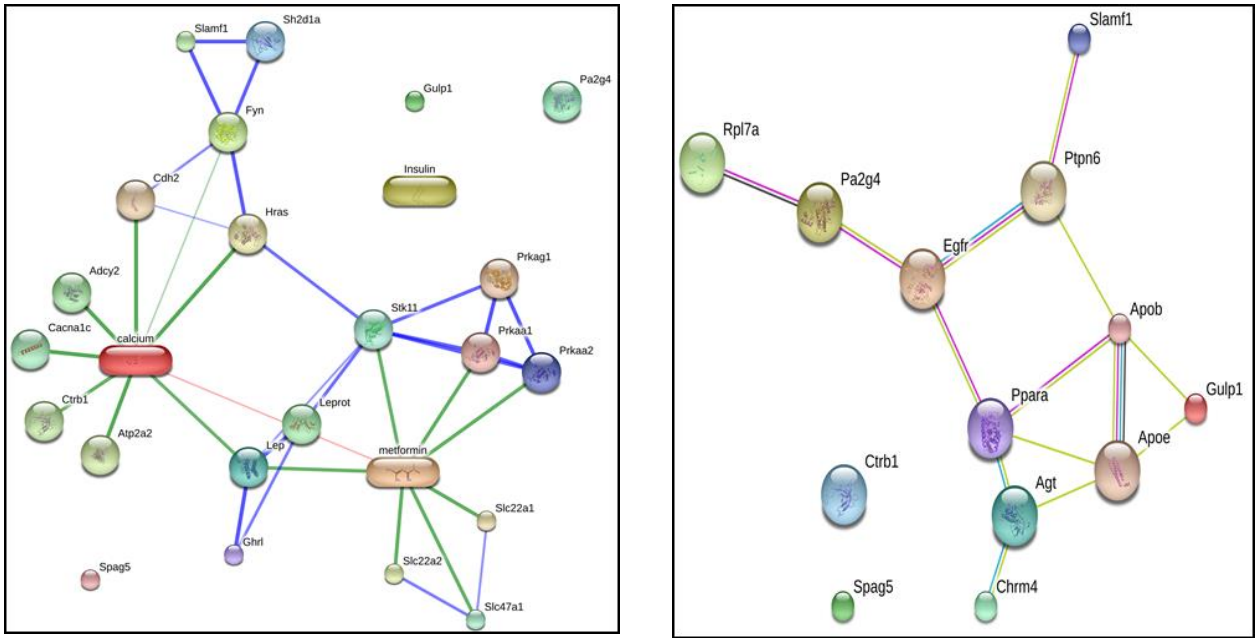

Figure 2: The protein interaction network analysis of the unique proteins in DM2C rat livers:
The STITCH chemical–protein interaction network for metformin and insulin. Metformin has been found to be linked to Slamf1 and Ctrb1 which are associated with type 2 diabetes. (b) The STRING protein–protein interaction network. Proteins Pa2g4, Slamf1, Gulp1, and Chrm4, which are the unique proteins of DM2C, showing functional interaction with other proteins associated with lipid metabolism via apolipoproteins, namely ApoB and ApoE. The different colors for the lines represent the different types of evidence for the association. Green color depicts gene neighborhood; red color: gene fusion; blue color: gene co-occurrence; purple color: experimental determination; and light blue color: evidence obtained from curated databases. The circular nodes indicate the different proteins.

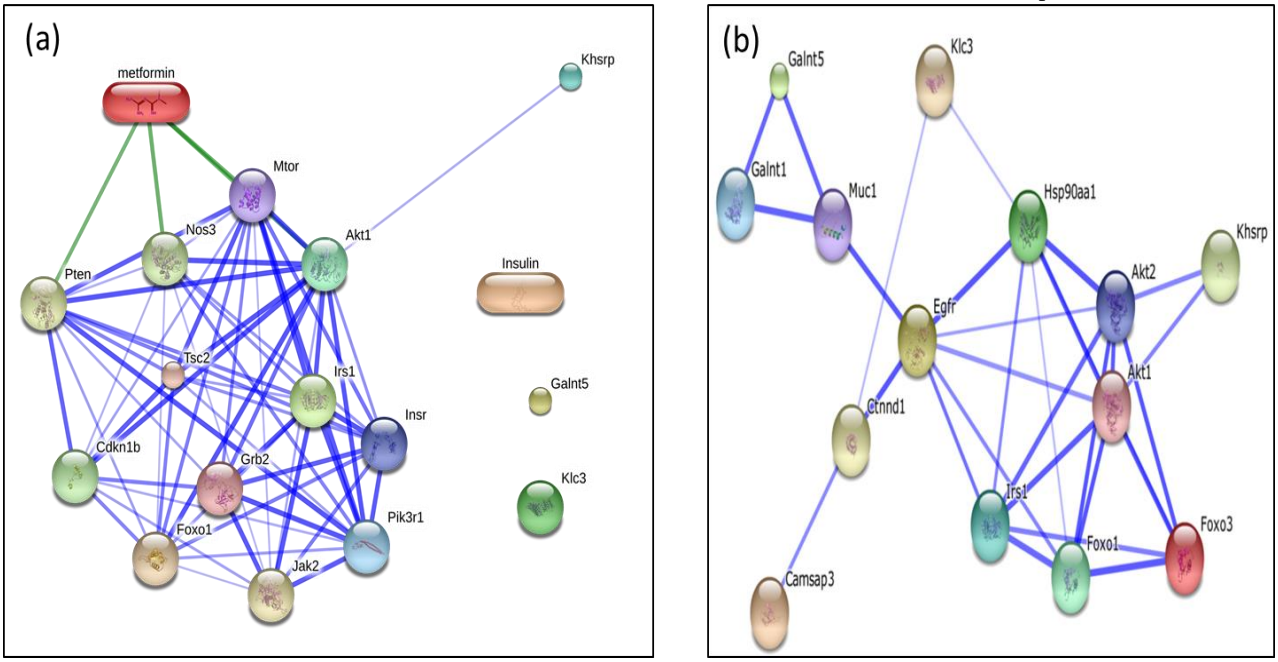

Figure 3: The protein interaction network analysis of the unique proteins in DM2-PR rat livers:
(a) The STITCH chemical–protein interaction network for metformin and insulin. Metformin has been found to be linked to Khsrp which is associated with type 2 diabetes. (b) The STRING protein–protein interaction network. The proteins Galnt5, Klc3, Khsrp, and Camsap3, which are the unique proteins in DM2-PR, showing functional interaction with other proteins associated with the insulin signaling pathway (Akt1, Akt2, Foxo1, and Irs1). The different colors for the lines represent the different types of evidence for the association. Green color depicts gene neighborhood; red color: gene fusion; blue color: gene co-occurrence; purple color: experimental determination; and light blue color: evidence obtained from curated databases. The circular nodes indicate the different proteins.

increased fasting insulin and fasting glucose means glucose cannot enter the cells even though the body responds with hyperinsulinemia. In addition to higher HOMA-IR, diabetic rats in our study showed insulin resistance which is the main characteristic of type 2 diabetes mellitus.

There are two types of diabetes: type 1 diabetes, or insulin-dependent diabetes, and type 2 diabetes, or non-insulin-dependent diabetes. Some proteins are expressed in type 1 diabetes and some are expressed in type 2 diabetes. In our study, by using the STITCH interaction network, it was found that the unique proteins in this study correlated with metformin and did not have interactions with insulin [Figure 2(a), Figure 3(a), and Figure 4(a)]. Therefore, we can conclude that the unique proteins in this study were exclusively associated with type 2 diabetes.

Calcium is one of the factors related with insulin resistance and insulin secretion. Calcium is involved in the phosphorylation of the insulin receptor, insulin signaling, insulin release from the pancreas, and GLUT4 translocation. Previously, some studies have shown that higher serum calcium levels are associated with incidence of type 2 diabetes[15]. In addition to this, serum calcium levels show a positive correlation with incidence of cardiovascular disease in patients with type 2 diabetes[16]. According to the STITCH interaction network, this was found true in this study. The unique proteins of type 2 diabetic rats (Ctrb1, directly; Slamf1, through Cdh2; and Fyn protein) are found to be associated with calcium.

Hypertriglyceridemia is one of the most common features of diabetes. Insulin deficiency or resistance leads to insufficiency of glucose intake into cells. Deficiency of glucose leads to increased fatty acid metabolism and an increase in free fatty acids. It makes more TG in the adipose tissue and muscle, and when serum triglyceride reaches the liver, VLDL will cause the triglyceride to be exposed more than its exposure under normal conditions[17]. ApoE mediates the clearance of VLDL from circulation, acting as a ligand for the hepatic lipoprotein receptor[18]. Some studies have suggested that hypertriglyceridemia is associated with delayed catabolism of Apo (apolipoprotein) B-containing lipoproteins. [19, 20] Interestingly, in our study, we found the network between our unique proteins (Gulp1, directly; Chrm4, via Agt; and Ppara proteins) expressed in diabetic rats and ApoE and ApoB. Considering this point, we can support the view that hypertriglyceridemia is the complication of diabetes which is involved in the micro and macro vascular complications of diabetes.

Liver is the main organ involved in glucose metabolism and glucose homeostasis. Hepatic insulin resistance is one of the most common pathological conditions of diabetes[3,4]. In this study, we found that there is protein interaction between the unique proteins of DM-PR (Galnt5, Klc3, Khsrp, and Camsap3) and the proteins involved in the insulin signaling pathway (Akt1, Akt2, Foxo1, and Irs1). In conclusion, it can be stated that our results suggest that purple rice might improve glucose metabolism via insulin signaling.

In type 2 diabetes, endoplasmic reticulum stress, oxidative stress, and lipotoxicity exist together to increase insulin resistance and worsen pancreatic β cell dysfunction. Reduction of oxidative stress is one of the relevant treatments for diabetes. The antioxidant system consists of numerous antioxidant compounds and several antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase. Superoxide dismutase, an antioxidant enzyme, is the main antioxidant enzyme for superoxide removal, and this is done by converting superoxide into hydrogen peroxide and molecular oxygen. In animal studies, the liver tissue of diabetic rats reveals significant reduction in both activity and protein expression of Cu Zn SOD, which leads to endoplasmic reticulum dysfunction[21,22]. Moreover, this protein has been linked to progressive diabetes nephropathy in C57BL/6-Akita and KK/Ta-Akita rats[23]. In addition, treatment with SOD has been found to reduce liver oxidative stress in diabetic rats and increase antioxidant enzyme activity[23,24]. In our study, we identified the SOD3 protein in normal rats and diabetic rats treated with purple rice, and absent in diabetic rats. We can conclude that purple rice may improve the condition of oxidative stress and thus bring about improved blood glucose control.

Autophagy is a cellular process for bulk degradation of cellular components that are damaged or functionally redundant. Autophagy-related proteins can be regarded as protectors of cells against cellular response to routine cell turnover[25]. A consequence of impaired autophagy is deposition of malfunctioning cellular organelles such as mitochondria within the cell. Mitochondrial dysfunction is associated with oxidative stress and leads to insulin resistance. Recent evidence has shown that impaired autophagy is related to obesity and diabetes. In animal models, reduction in hepatic autophagy may underline the insulin resistance by down-regulation of autophagy-related proteins, LC3, Beclin1, ATG5, and ATG7[26,27]. In addition to this, inhibition of autophagy may contribute to the pathogenesis of the diabetic heart and nephropathy[28-30]. In our study, we identified that the Beclin1 protein increased in diabetic rats with purple rice treatment. Beclin 1 is an autophagy-related protein, with downstream signaling to the anti-apoptosis protein, Bcl2[29]. From our results, we can conclude that purple rice treatment may regulate autophagy of cells and may contribute to reversal of diabetes status.

In conclusion, type 2 diabetes is one of the commonest metabolic diseases all over the world. Dyslipidemia is associated with the diabetes in most diabetic patients. In our study, the association of dyslipidemia with diabetes could be proved because the study found that the unique proteins of diabetic rats had interactions with the lipoprotein metabolism. Peripheral insulin resistance is a major pathological condition of type 2 diabetes. The present study found that improving insulin signaling is one of the effects of purple rice. Hyperglycemia leads to oxidative stress, and this oxidative stress is recovered by purple rice with the increased amounts of oxidative stress proteins (SOD) in the purple rice treatment group. In addition to this, purple rice is also involved in the autophagy mechanism, which is a mechanism that protects cells from cell death. In conclusion, our study can support

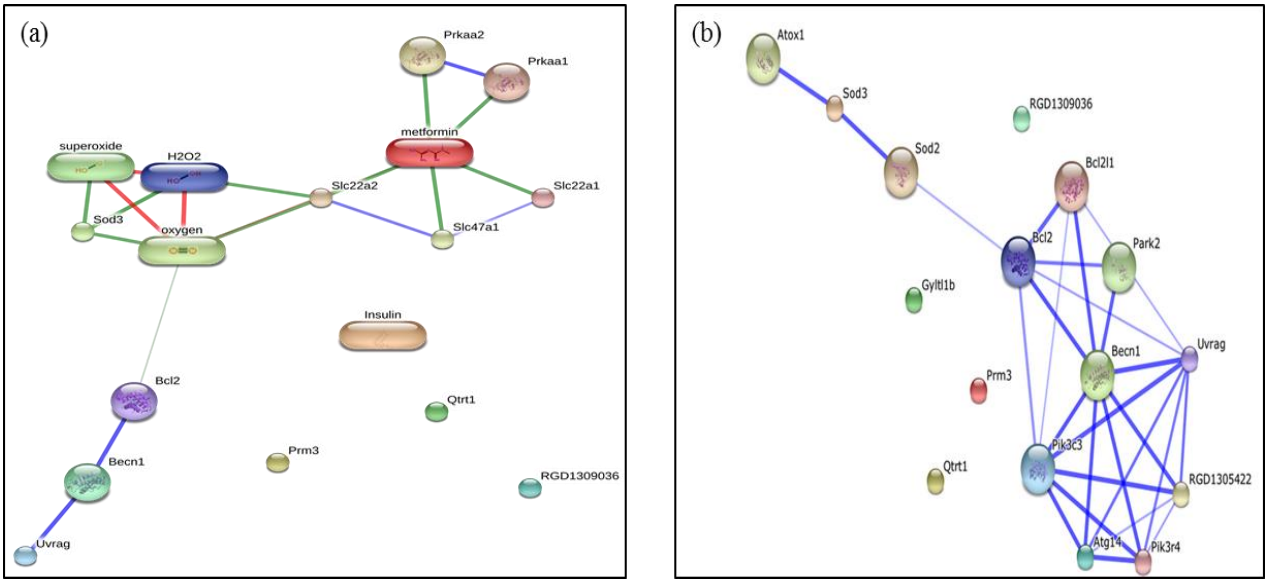

Figure 4: The protein interaction network analysis of shared proteins in NC and DM2-PR rat livers: The STITCH chemical–protein interaction network for metformin and insulin. Metformin has been found to be linked to Becn1 and SOD3 which are associated with autophagy and antioxidant activity. (b) The STRING protein–protein interaction network. The proteins Becn1 and SOD3, which are the shared proteins of normal and type 2 diabetic rats with purple rice bran supplementation, showing functional interaction with other proteins associated with antioxidant activity (SOD2) and autophagy (Atg14, Pik3r4, and Pik3c3). The different colors for the lines represent the different types of evidence for the association. Green color depicts gene neighborhood; red color: gene fusion; blue color: gene co-occurrence; purple color: experimental determination; and light blue color: evidence obtained from curated databases. The circular nodes indicate the different proteins.

the existing knowledge about dyslipidemia with regard to diabetes. Moreover, this study is one more step forward in the direction of better understanding the anti-hyperglycemic effect of purple rice via insulin signaling, oxidative stress response, and autophagy.

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