ISSN: 0976-3104
SUPPLEMENT ISSUE

ARTICLE

A COGNITIVE STUDY TO EVALUATE ANTIHYPERGLYCEMIC PROPERTY OF ORYZA SATIVA GLUTINOSA ON SPRAGUE DAWLEY

Prasetyorini Djarot*, Mulyati, Faulenly
Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Pakuan, Bogor, INDONESIA

ABSTRACT

Current study aims at *evaluation of the anti-hyperglycemic property of Oryza Sativa glutinosa on Sprague Dawley. Initially 20 male rats (Sprague-Dawley spp.) to eliminate error that may ensue due to cyclic hormonal variation in female rats in pregnant and non-pregnant states, induction of diabetic state was done by administering 150mg/bb of alloxan intraperitoneally until the blood sugar level rose to ≥200 mg / dL upon which Sprague-Dawley were divided into 5 groups. The treatments were positive control (metformin 0.012 g), negative control (distilled water), 1 dose of black glutinous rice (0.53 g), dose 2 black glutinous coffee (0.27 g), and dose 3 black glutinous coffee (1.06 g). Results: Changes in body weight and blood sugar levels were evident from day 3 of therapy, black glutinous coffee at dose 1.06 g / 200 g BW could decrease blood glucose level by 58% from the baseline readings (271.75 mg / dL to 114.25 mg / dL) within 12 days of treatment (P<0,01) Black glutinous rice (O. sativaglutinosa) proved to be antihyperglycemic on Sprague Dawley's male rat. (P<0, 01). The prognosis of diabetes is highly dependent on weight and blood sugar control. The potency of Oryza sativa glutinosa in a reduction of blood sugar levels as well as its efficiency in weight reduction on Sprague-Dawley makes it a promising drug in the management of diabetes. The drug is also cost effective and tolerable making it accessible to many and has the potential of modifying the current rising trend of diabetes mellitus type 2 globally.*

KEY WORDS
Antihyperglycemic, coffee, black gluteous rice, Sprague dawley rats, diabetes mellitus

INTRODUCTION

Diabetes mellitus is a complex metabolic disorder, involving abnormalities of insulin secretion and insulin action, and causes glucose intolerance and hyperglycemia [1]. According to 2011 International Diabetes Federation statistics out of the 451 million people who have diabetes worldwide an estimate of 153 million people were from the 21 West Pacific members of which Indonesia is a member corresponding to 34% of global diabetes burden. Diabetes contributes to 43% of all premature deaths, 80% end stage renal disease and thrice increases the risk of cardiovascular disease. About 10 million of diabetes cases was documented in Indonesia in 2015[2], The rise in global prevalence of diabetes (422million in 2010 to 451 million cases in 2015), pose a significant threat to developing healthcare systems like Indonesia with regard to direct health care costs, disability and time.3 About $4.47 trillion was used in the management of diabetes in terms of medications and emergency care in 2015[3]. Over the years due to rising incidence of diabetes significant increase in the use of oral anti-hyperglycemic agents and insulin without meeting the recommended glycemic target has been reported. According to the WHO 2016 statistics, the prevalence of diabetes in Indonesia is 6.6% in males and 7.3% in females [4]. The prevalence of diabetes in Indonesia is 4.4% and 10.2% for glucose intolerance among the urban productive age groups consisting of 3.5% undiagnosed cases[. Obesity contributes to 68.7% of diabetes cases with 42.7% having central adiposity diabetes comorbidities in adult age group in Indonesia consist of hypertension contributing to more than 40% of cases and Dyslipidemia occurs in more than 50% of diabetic patients. The rise in risk factors, for instance, obesity 31.4% and physical inactivity 22.8% in addition to sedentary lifestyles have shown a direct link to the rise in diabetes burden in both urban and rural populations [5].

Received: 3 May 2017
Accepted: 5 June 2017
Published: 12 Aug 2017

In 1994 there were 2.5 million DM patients in Indonesia and is expected to raise about 5 Million by 2020. In recent years, there has been increasing attention to the role of plant bioactive components in the treatment of diabetes mellitus. Brown rice can reduce the risk of type 2 diabetes; this is because the Aleurone found in brown rice can increase glucose metabolism [6]. Other bioactive contents which can decrease blood glucose levels include flavonoids [7]. Flavonoids can be found in the anthocyanin pigment contained in black glutinous rice [8].

Based on its flavonoid, black glutinous (*Oryza sativa glutinosa*) allegedly has anti-hyperglycemic properties. People consume black glutinous rice in the form of porridge, so its bioactive component decreases. According to previous research black glutinous rice prepared by steeping can preserve the bioactive ingredient [9]. However, at the moment only a few people know the benefits of black glutinous rice coffee, so there are still many who consume coffee. According to Suhartatik, consuming more than 5 cups of caffeine coffee per day will increase the occurrence of damage to blood vessel walls [9]. This is very risky for people with diabetes mellitus who have a higher risk of cardiovascular disease, so it is necessary to find another alternative. Based on the research conducted empirically, consuming 30 g of black glutinous rice brewed with hot water per day can lower blood glucose levels for people with diabetes mellitus, and of course, this steeping is free of caffeine ingredient. This needs to be tested through scientific research to establish the most efficient dose of black sticky rice as an anti-hyperglycemic agent so it can be used as a substitute for coffee for people with diabetes mellitus.

***Corresponding Author**
Email:
prastyorinidjarot@gmail,com
Tel.: +62-81298036348
Fax: +62-2518375547

MATERIALS AND METHODS

The materials used in this study are black glutinous rice , 20 male white rats Sprague Dawley, 70% ethanol and 95% distilled water, 512 type pellets for rat feed, alloxan, metformin, 10% gelatin solution, magnesium powder (Mg), 2 N (HCl) hydrochloric acid, and 10% sodium chloride (NaCl). The tools are used in this research include gloves, masks, injections, oral sonde, scales, er Len Mayer, Brown bottles, watch glass, measuring flask, test tube, measuring cup, beaker, stirrer, spatula, Wooden clip, porcelain bowl, funnel, mouse cage, refrigerator, water bath, blender, easy touch, and glucometer strips. Current research used 20 male Sprague-Dawley rats aged 3 to 4 months old and weighed approximately 200g. The treatment group of 5 each were made consisting of 4 rats in each group. The tested groups were separated in a cage of a plastic box and a wired cap. The cage was floored by husks and to be replaced every two days once the cage condition remains dry. During the study, all groups of rats were fed pellet type 512 and drank water daily. Weight assessment and measurement of the number of consumptions was done every day. All the experimental animals were treated for 12 days.

Preparation of black sticky coffee

A total of 30 g of black glutinous rice was washed and drained.
Then it was roasted until the smell, and the color became darker. Then the black glutinous rice is mashed by a blender. The black sticky rice powder is sieved to obtain a fine powder. The black glutinous powder is brewed using boiling water, then administered orally according to the given dose. Treatment was given for 12 days after known alloxan induction of hyperglycemic state.Induction of hyperglycemic state in healthy mice that have been fasted for 10-12 hours was done by giving alloxan with a dose of 150 mg/kg BB intra peritoneal. Blood sugar levels were measured before alloxan administration (day 0) and after alloxan administration (day 3). Only mice with blood sugar content of 200 mg / dL were used in this study. The sample that attained blood sugar levels of ≥200 mg / dL was divided into 5 treatment groups, namely:

1. The treatment I: Positive controls gave metformin 0.012 g in 1 ml distilled water.
2. Treatment II: Negative controls given only 1 ml of distilled water.
3. Treatment III: (Dose I) Black glutinous coffee with a dose of 0.53 g in 1 ml of distilled water.
4. Treatment IV: (Dose II) Black glutinous coffee with a dose of 0.27 g in 1 ml distilled water.
5. Treatment V: (Dose III) Black glutinous coffee with a dose of 1.06 g in 1 ml of distilled water.

Each group of mice received different treatment dose, for 12 days. Blood glucose measurements were performed on days 3, 6, 9, and 12.

Parameter of research

1. Blood Sugar
Levels Measurement of blood sugar levels was done using Easy Touch tool. A blood sample was obtained by the pricking the tail followed by dripping on strips which were then installed on the tool Accu. Blood glucose levels were expressed in mg / dL.
2. WeightThe analytical weighing machine was used to monitor the weight of the rats.
3. Dietary intake During the study, all groups of rats were given food and drinking water. Meals were measured to ensure uniformity.
4. Active ingredientTo identify the active ingredient in black glutinous rice phytochemical tests which include flavonoid test, saponin test, tannin test, and terpene test was done.

Phytochemical test

Flavonoid test
Black glutinous powder of 0.5 g was dissolved in 3 ml of 95% concentrated ethanol from which 2 ml of black glutinous powder and ethanol mixture is added to 0.1 g of magnesium powder. The above preparation is shaken gently by adding 10 drops of concentrated hydrochloric acid (HCL). The orange-red to red-purple that formed showed positive flavonoids. [10]

Saponin test
Black glutinous rice powder as much as 0.5 g is put into the test tube, added 10 ml of hot distilled water, cooled it and shaken it for 10 seconds. Positive results are characterized by the formation of foam that is not less than 10 minutes and as high as 1 to 10 cm. Also, 1 drop of hydrochloric acid 2 N foam is not lost [10].

Tannin test
Black Black glutinous rice powder of 0.5 g was dissolved in 3 ml of distilled hot water and stirred after cooling and centrifugation, 10% 0f sodium chloride solution was added and filtered. The filtrate of 1 ml was added to 10% gelatin solution, the presence of precipitate signified a positive result.

Penetration test

0.5 g of black glutinous powder was dissolved in 2 ml of chloroform followed by addition of 3 ml of concentrated sulfuric acid carefully. The formation of reddish brown color on the surface of the solution is a positive test for the presence of terpenoids [11].

Research Design (Data Analysis): To get a conclusion from the study, the data obtained was analyzed by using Data analysis was done using SPSS 16.0 software, then described as the average which was then presented in table form for easy comparison. In the comparison of treatment outcomes, a t-test was used to compare the difference between the study sample, the negative and the positive control. Paired t test was used relate data before and after treatment. All P values of less than 0,001 were considered statistically significant

RESULTS

Phytochemical test results

The phytochemical test is one of the necessary steps in exposing the potential of plant resources, particularly to know the compounds in it. The results of phytochemical screening can be seen in following [Table 1].

Table 1: Phytochemical test results

Chemical Test	Reactants	Physical Indicators	Value
Flavonoid	Magnisium powder and HCl	P	+
Saponin	*Aquadest*	Froth formed	+
Tanin	$FeCl_3$ 3%	Bownish brownish gree or blackish blue colour	+
Terpen	Chlorofom and HCl	Forms reddidsh brown colour	+

Black glutinous rice showed positive results on flavonoids, saponins, tannins, and terpene. On the flavonoid test, positive results are indicated by orange color; this is due to the reduction of flavonoids with magnesium (Mg) producing complexes with red or orange color. In the saponin test, the positive results were characterized by the formation of foam [10], In tannins positive results was marked by the formation of brownish green or Blue foam, on addition of 3% FeCl3 reagents to the sample, it reacts phenolic moiety of the tannin compound. In the terpene test positive results is characterized by the formation of a reddish-brown foam, due to the oxidizing effects of HCL[11]. Alloxan Induction of hyperglycemic states in Sprague-Dawley Intraperitoneal alloxan treatment (IP) with a dose of 150 mg/kg was able to increase blood glucose levels of rats by 169.25% from 102.50 mg / dL to 271.75 mg / dL, listed in [Table 2]

Table 2: Percentage of blood glucose level prior to Alloxan induction

Gro up	Day 1	Day -3	Percentage rate
1	102.25 ±	271.75	169.50 ±
	2.63	± 34.33	31.70
	102.75 ±		
2	3.20	271.75	169.00 ±
	3.20 103.00 ±	± 32.60	29.40
3	2.16	271.75	168.75 ±
	2.16 102.25 ±	± 35.48	33.32
4	3.86	271.75	169.50 ±
	3.86 102.25 ±	± 11.47	7.613

5	3.86	271.75	169.50 ±
	3.86	± 32.34	28.48
Approxi mately	3.14	271.75	169.25 ±
		± 29.24	26.10

Intraperitoneal (IP) treatment was the preferred route in this test due to the advantages of intraperitoneal treatment over the abdominal cavity infiltration. Intraperitoneal (IP) route is attributed to the rapid onset of Alloxan action on the target pancreatic β-cells compared to the intra-abdominal route. During the study, changes in body weight were used as one of the markers of drug efficacy [12].

The average increase in body weight after induction with Alloxan was recorded and taken as the baseline value for follow up [Table 3].

Table 3: Body weight before induction of Alloxan

Group	Day 1	Day 3	Rate of increase
1	236.75 ±	253.75	17.00 ±
	8.65	± 8.09	0.56
2	238.00 ±	256.00	18.00 ±
	8.12	± 2.44	5.68
3	235.75 ±	255.75	20.00 ±
	7.13	± 5.32	1.81
4	236.75 ±	254.00	17.25 ±
	9.94	± 4.89	5.35
5	236.75 ±	253.75	17.00 ±
	39.94	± 6.75	3.19
Average rate	236.80 ±	254.65	17.85 ±
	8.82	± 5.49	3.32

Based on the above results, the increasing in body weight occurred on the third day after alloxan induction.

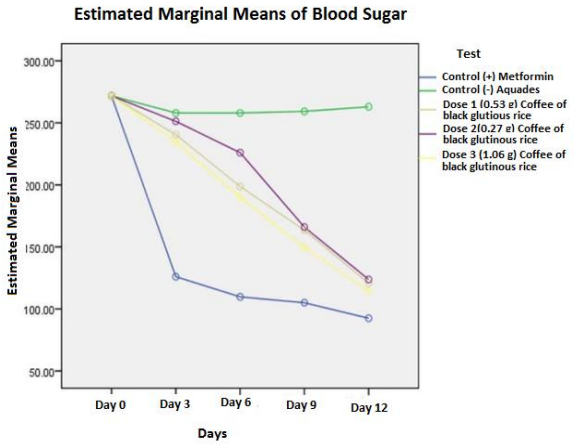

Fig. 1: Graph of average blood glucose level in S dawley.

Obesity is one of the factors that influence the incidence of diabetes mellitus type 2. Visceral adiposity, for instance, is associated with impaired leptin signaling and peripheral insulin resistance that affects blood glucose levels in people with diabetes mellitus [13].

The Effect of Black glutinous rice Coffee on Blood Glucose Level

The results of black glutinous coffee activity test as the anti-hyperglycemic agent in experimental mice are presented in graphical form in [Fig. 1].

Based on the graph, decrease in blood glucose levels of rats started on the 3rd day after black glutinous rice was given.

In the positive control group given metformin, a significant decline in blood glucose levels on day 3, followed by a decrease in blood glucose levels to baseline on day 12 is observed. The reduction in blood glucose level in the positive control is due to metformin action through increase glucose uptake by tissues. Whereas in negative control the anti-hyperglycemic effects are due to physiological action of insulin in response to high blood sugar.[14]

[Table 4] shows that giving black glutinous rice at a dose of 1 (0.53 g), a dose of 2 (0.27 g), and a dose of 3 (1.06 g) can lower blood glucose levels in rats significantly (P<0, 01). The decrease in blood glucose levels was noticeable on day 3 of black glutinous rice. However, blood glucose levels were still high when compared with positive controls. Decreased of blood glucose levels are also caused by secondary metabolite compounds contained in black sticky rice.

Flavonoids have properties as antioxidants that provide protective properties against insulin- producing cells- β flavonoids also act via inhibition of glucose transporter isoform 2 (GLUT2), intestinal transporters for glucose. According to Arulselvan saponin compounds can regenerate the pancreas to increase the number of β-cells [6, 13]. Tannins enhance glucose uptake and inhibit adipogenesis in 3T3-L1 adipocytes through PTP1B inhibition modifying disease in peripheral insulin resistance. Liu et al show that tannins increases blood glucose transport by activating insulin-mediated signaling pathways. Terpenoid compounds also exhibited antidiabetes action through inhibition of the α-glucosidase enzyme [15, 16].

Table 4: Bloog Glucouse content (mg/dL) in S dawley during the test

Test	Day 1	Day 3	Day 6	Day 9	Day 12	Average
Positive control	271.75±34.33	126.00±32.60	109.75±8.81	105.00±6.00	92.50 ±7.51	141[a]
Negative control	271.75±32.60	258.00±35.28	258.00±35.28	259.25±34.76	263.00± 35.39	262[d]
Dose 1 (0.53 g)	271.75±35.40	240.50±19.64	198.75±11.5	163.50±5.51	120.75± 0.96	199.05 [b,c]
Dose 2 (0.27 g)	271.75±11.47	251.25±6.08	226.00±3.55	161.00±2.31	123.75± 1.71	207.75 [c]
Dose 3 (1.06 g)	271.75±32.34	234.50±29.72	190.00±22.64	149.75±16.46	114.25± 2.87	192.05 [b]
Average	271.75[e]	222.05[d]	196.50[c]	168.70[b]	142.85[a]	

Explanation: the number followed by different superscript in each column or rows show significant variations in blood sugar levels. (P<0.01).

Total dietary intake during the Study

During the acclimatization period, all animal groups were fed pellet of 512 types as much 100 g per day. After the induction of alloxan and blood glucose levels is ≥ 200 mg / dL, The amount of feed consumption was 110 g per day, it aims to see the effect of feed consumption when blood glucose rises. Based on this research it was concluded that when the blood sugar increased feed, consumption was also increased, as people with diabetes mellitus tend to experience symptoms of polyphagia and dysfunction in leptin-satiety pathway, especially in visceral obesity [14].

The effect of black glutinous rice coffee on body weight

In [Table 5], it can be seen that the lowest body weight is seen in the group of positive control treated rats given metformin that is equal to 227.75 g. As metformin is associated with a reduction in appetite in diabetes type II and cannot increase body weight [20]. Furthermore, the lowest body weight is also seen in the rats that were given black glutinous rice at a dose of 3 (1.06 g) it is 237.75 g. The high content of fiber in black sticky rice provides longer safety effects hence suppressing the addition of body weight. The highest body weight was seen in the group of negative control treatment rats corresponding to 261.25 g.

[17]

Table 5: The weight of rats (g) during treatments

Treatment	Day 1	Day 3	Day 6	Day 9	Day 12	Rata-rata
Positive control	253.75±8.09	250.00±5.72	247.25±2.98	240.00±2.22	227.75±2.11	244.45[a]
Negative control	276.00±2.44	245.75±2.21	256.75±2.22	259.00±1.58	261.25±1.58	257.55[c]
Dose 1 (0.53 g)	255.75±5.32	252.75±5.31	250.25±5.12	247.50±4.20	244.00±4.09	249.50[b]
Dose 2 (0.27 g)	254.00±4.89	252.25±4.50	250.00±4.24	247.50±4.20	244.75±4.03	249.70[b]
Dose 3 (1.06 g)	253.75±6.75	249.75±29.72	245.75±6.75	241.75±6.75	237.75±6.75	245.75[a]
Rata-rata	254.65[d]	252.45[c,d]	250[b,c]	247.25[b]	242.60[a]	

Explanation: the number followed by different superscript in each column or rows show significant variations from baseline body weight (P<0.01).

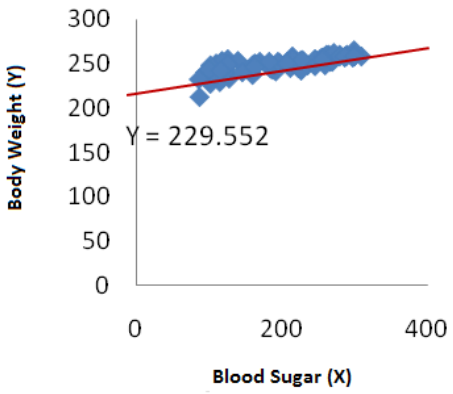

Fig. 2: Graph of correlation between blood glucose levels with animal body weight test.

The above [Fig. 2] explains that the relationship between blood glucose level and body weight can be expressed in the equation Y = 229.52 + 0.99x, meaning that each addition of 1 g of body weight will increase blood glucose level of 0.099 mg / dL. Obesity is one factor that affects the incidence of diabetes mellitus type 2. Excessive fat deposits in the body can cause insulin resistance that affects blood glucose levels in people with diabetes mellitus. So the above regression equation can be modeled to determine the effect of body weight on certain blood sugar levels [5].

CONCLUSION

The prognosis of diabetes is highly dependent on weight and blood sugar control. The potency of *Oryza sativa glutinosa* in the reduction of blood sugar levels as well as its efficiency in weight reduction on Sprague-Dawley makes it a promising drug in the management of diabetes. The drug is also cost effective and tolerable making it accessible to many and has the potential of modifying the current rising trend of diabetes mellitus type 2 globally. Further research is needed to evaluate the efficacy and tolerability of *Oryza sativa glutinosa* in diabetic patients in order to achieve lower blood glucose level that meet the positive control level.

CONFLICT OF INTEREST
There is no conflict of interest.

ACKNOWLEDGEMENTS
We appreciate Ferdowsi university of Mashhad and Muniplicity, parks organization for technical and financial help.

FINANCIAL DISCLOSURE
The study was funded by Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Pakuan, Bogor, Indonesia

REFERENCES

[1] Hartoyo A, Muchtadi D, Astawan M, Dahrulsyah WA. [2011] Pengaruh ekstrak protein kacang komak (Lablab pupures (l.) Sweet) pada kadar glukosa dan profil lipida serum tikus diabetes. Jurnal teknologi dan industri pangan. 1(22):1-6.

[2] Whiting DR, Guariguata L, Weil C, Shaw J. [2011] IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes research and clinical practice. 94(3):311-321.

[3] Soewondo P, Ferrario A, Tahapary DL. Challenges in diabetes management in Indonesia: a literature review. Globalization and health. 2013 Dec 3;9(1):63.

[4] Akbar MF, Ilmi YH. View point The use of insulin for early diagnosed patients with type 2 diabetes mellitus in primary health care in Indonesia: a general practitioner's perspective.2013

[5] World Health Organization (WHO). Diabetes country profiles, 2016.

[6] Lukačínová A, Mojžiš J, Beňačka R, et al. [2008] Preventive effects of flavonoids on alloxan-induced diabetes mellitus in rats. Acta Veterinaria Brno. 77(2):175-182.

[7] Song J, Kwon O, Chen S, Daruwala R, Eck P, Park JB, Levine M.[2002] Flavonoid inhibition of sodium-dependent vitamin C transporter 1 (SVCT1) and glucose transporter isoform 2 (GLUT2), intestinal transporters for vitamin C and glucose. Journal of Biological Chemistry. 3;277(18):15252-15260.

[8] Suhartatik N, Cahyanto MN, Raharjo S, Rahayu ES. [2013] Antioxidant activity of anthocyanin of black glutinous rice during fermentation. Jurnal Teknologi dan Industri Pangan. 24(1):115-119.

[9] Zhang P, Zhang X, Brown J, Vistisen D, Sicree R, Shaw J, Nichols G. [2010]Global healthcare expenditure on diabetes for 2010 and 2030. Diabetes research and clinical practice. 87(3):293-301.

[10] Indonesia DK. Materia Medika Indonesia. Jakarta: Departemen Kesehatan Republik Indonesia. 1995.

[11] Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African journal of biotechnology. 2005;4(7):685-688.

[12] Rohilla A, Ali S. [2012] Alloxan induced diabetes: mechanisms and effects. International journal of research in pharmaceutical and biomedical sciences.3(2):819-823.

[13] Khan A, Faheem M, Shah ST, et al.[2015] Frequency of abdominal obesity and its association with diabetes mellitus among people of peshawar. Journal of Ayub Medical College Abbottabad. 30;27(3):617-619.

[14] Shaw JE, Sicree RA, Zimmet PZ.[2010] Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes research and clinical practice. 31;87(1):4-14.

[15] Muthusamy VS, Anand S, Sangeetha KN, Sujatha S, Arun B, Lakshmi BS. [2008]Tannins present in Cichorium intybus enhance glucose uptake and inhibit adipogenesis in 3T3-L1 adipocytes through PTP1B inhibition. Chemico-biological interactions. 10;174(1):69-78.

[16] Arulselvan P, Ghofar HA, Karthivashan G, Halim MF, Ghafar MS, Fakurazi S. Antidiabetic therapeutics from natural source: A systematic review. Biomedicine & Preventive Nutrition. 2014 Dec 31;4(4):607-17.

[17] Liu X, Kim JK, Li Y, Li J, Liu F, Chen X.[2005] Tannic acid stimulates glucose transport and inhibits adipocyte differentiation in 3T3-L1 cells. The Journal of nutrition. 135(2):165-171.