Food Research 8 (Suppl. 6) : 46 - 50 (2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Effect of combination of germinated brown rice and oyster mushroom on the inflammatory response and glycemic control in hyperglycemic aged rat model

[1]Andarini, S., [2,*]Maulidiana, A.R., [2]Rahmawati, I.S. and [2]Handayani, D.

[1]*Department of Public Health and Preventive Medicine, Faculty of Medicine, Universitas Brawijaya, Indonesia*
[2]*Department of Nutrition, Faculty of Health Sciences, Universitas Brawijaya, Indonesia*

Article history:
Received: 13 August 2023
Received in revised form: 20 August 2024
Accepted: 23 November 2024
Available Online: 16 December 2024

Keywords:
Germinated brown rice,
Oyster mushroom,
Inflammatory response,
Serum glucose,
Insulin resistance

DOI:
https://doi.org/10.26656/fr.2017.8(S6).11

Abstract

The elderly are a high-risk age group that is more vulnerable to severe diseases, such as diabetes, cardiovascular disease, and respiratory tract infections. Germinated brown rice (GBR) has glucose-lowering properties and antioxidant potential due to high phenolic bioactive compounds. Oyster mushroom (*Pleurotus oestreatus*) is rich in β-glucans with its wide-known anti-inflammatory effect. This present study evaluated the effect of the combination of GBR and oyster mushroom supplementation in the inflammatory response and glycemic effect on aged rats. Supplementation was given at 250 mg per kg body weight per day in three different ratios and administered to fructose-induced hyperglycemic aged rats for 8 weeks. A total of thirty-five rats aged 12-18 months were randomly assigned into five groups, negative (I) and positive (II) control, GBR: oyster mushroom ratio extract of 60%:40% (III), 70%:30% (IV), and 90%:10% (V). Groups II to IV received 50% fructose in the drinking water. The inflammatory and glycemic responses were evaluated by measuring serum TNF-α, IL-6, fasting glucose and insulin. HOMA-IR and HOMA-β were also calculated. All supplementation formulations had not been able to show a significant reduction in the levels of pro-inflammatory markers as well as fasting insulin markers. However, this formulation was able to reduce the serum glucose level ($p = 0.001$) and insulin resistance level ($p = 0.002$) of hyperglycemic rats to a similar level as the negative control rat. No significant difference was found between the three different formulations. In summary, the combination of GBR and oyster mushroom supplementation has the potential as functional food for the management of hyperglycemia and its possible complications.

1. Introduction

Physical and physiological weaknesses are traits of aging in humans (Weyand *et al*., 2014). Aging is linked to declines in body function, including adaptive and innate immunity. As a result, elderly people are more prone to infections and autoimmune diseases with the coexistence of multiple chronic diseases. Another significant aspect of aging is increased pro-inflammatory cytokines (TNF-α and IL-6) levels which develop a low-grade chronic inflammation (de Almeida Brasiel, 2020).

During the coronavirus disease (COVID-19) pandemic, the infection was found in all age groups, however, some groups were reported to have a higher risk of morbidity and mortality such as the elderly; individuals with comorbidities, such as diabetes mellitus, hypertension, heart disease; and immune system disorders (Rothan and Byrareddy, 2020). In the elderly aged seventy years or older, the incubation period may be shorter due to a weak immune response (Qin *et al*., 2020; Shi *et al*., 2020; Tay *et al*., 2020). Therefore, the prevention and control of the coronavirus pandemic focuses on strengthening the body's immune system, such as the body's protection against bacteria, viruses, and other disease-causing agents. Nutrition is a potential therapeutic for COVID-19 (de Almeida Brasiel, 2020), by means of nutritional management, functional food, or supplementation.

Germinated brown rice (GBR) is a widely known functional food that has various health benefits. GBR has a lower carbohydrate percentage and more fiber content than white rice; thus, has a lower glycemic index (Sekar and Ayustaningwarno, 2013). Additionally, its content of GABA (gamma-aminobutyric acid) and phenolic bioactive compounds is higher than other types of rice so

*Corresponding author.
Email: *annisarizky@ub.ac.id*

eISSN: 2550-2166 /
Published by Rynnye Lyan Resources

it has antioxidant activity with a more optimal immune-boosting function (Ravichanthiran *et al*., 2018; Munarko *et al*., 2020). White oyster mushroom (*Pleurotus ostreatus*) has a good protein content and complete essential amino acids. Its functional component, β-glucan, is useful as an antioxidant, protection against viral infections, and anti-inflammatory (Pranamuda *et al*., 2012; Tjokrokusumo, 2018).

During the COVID-19 pandemic, functional food innovation is very indispensable, specifically for the elderly to improve their immune system and overall well-being, and simultaneously reduce the risk of degenerative diseases. The aim of this study was to investigate the effect of the combination of GBR and white oyster flours on the level of IL-6, TNF-α, and insulin resistance in a fructose-induced hyperglycemic aged rat model.

2. Materials and methods

2.1 Study design

This study was true experimental laboratory research using a randomized posttest-only control group design. The subjects were randomized using a simple random sampling method. Germinated Brown Rice and white oyster mushroom flours were mixed with three different formulations, with ratio of 90%:10%, 70%:30% and 60%:40% (w/w).

2.2 Test animals

A total of 35 male Wistar strain white rats (*Rattus norvegicus*) aged 12 to 18 months old to mimic the old age of humans was used in this study. The cage was made from polypropylene sized 7 × 9.5 × 7 inches. Rats were given standard normal rat feed and ad libitum access to water. For treatment groups, groups III, IV, and V, the water was mixed with 50% fructose solution to trigger the metabolic syndrome. All rats were grouped as follows:

Group I (Negative control) = normal rats, normal diet

Group II (Positive Control) = normal diet + 50% fructose solution

Group III (Treatment 1) = normal diet + 50% fructose solution and GBR flour supplement: oyster mushroom dose 1 (60:40)

Group IV (Treatment 2) = normal diet + 50% fructose solution and GBR flour supplement: oyster mushroom dose 2 (70:30)

Group V (Treatment 3) = normal diet + 50% fructose solution and GBR flour supplement: oyster mushroom dose 3 (90:10)

The study subjects were kept in a room with an optimal temperature (22-24°C), 50-60% humidity, and a 12:12 light-dark cycle on a regular basis. The experimental procedures were approved and carried out in accordance with the protocol of the Animal Ethics Committee, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia (No. 65/EC/KEPK/02/2021).

2.3 Laboratory analysis

Serum TNF-α, IL-6, and insulin levels were determined using the ELISA kit (BT-Lab, South Korea). The samples were read using a spectrophotometer at OD 450 nm. Meanwhile, blood glucose level was measured using glucose oxidase-phenol 4-aminoantipyrine (GOD-PAP) enzymatic method (Dia Sys) and read at a wavelength of 500 nm.

2.4 Insulin resistance measurements

Insulin resistance is a clinical condition that shows the low potential of endogenous and exogenous insulin to increase the uptake and use of glucose by body cells. Insulin resistance was calculated based on the equation by Anwer (2014):

$$\mathrm{HOMA-IR} = \frac{[\text{fasting insulin (mIU/mL)} \times \text{fasting glucose(mg/dL)}}{405}$$

To measure the strength level of pancreatic beta cells that produce insulin, HOMA-β% was calculated as:

$$\mathrm{HOMA-\beta\%} = \frac{[360 \times \text{fasting insulin (mIU/mL)}]}{[\text{fasting glucose(mg/dL)} - 63]}$$

2.5 Statistical analysis

All data was statistically analyzed by SPSS for Windows. Data was checked for normality and homogeneity of variance. Multiple comparisons were performed either by one-way ANOVA followed by post-hoc Tukey test for normal distribution data or Kruskal-Wallis followed by Mann-Whitney for not-normally distributed data. Statistical significance was considered as a 0.05 significance level and a 95% confidence interval.

3. Results and discussion

This study aimed to determine the optimum ratio of GBR and oyster mushrooms to improve inflammatory response and glycemic control of old rats during 8 weeks of treatment. This experimental animal study used elderly mice with 50% fructose solution to induce metabolic disorders. The mean levels of TNF-α, IL-6, insulin, and fasting blood sugar in rat serum after the intervention are presented in Table 1.

Based on Table 1, the lowest mean of TNF-α level was in group V while the highest average was in group

https://doi.org/10.26656/fr.2017.8(S6).11

Table 1. Mean serum concentrations of TNF-α, IL-6, insulin, and glucose.

Group	N	TNF-α (ng/L)	IL-6 (ng/L)	Insulin (mIU/L)	Glucose (mg/dL)
I	7	96.65±19.40	4.26±1.67	4.98±1.29	83.86±8.42[a]
II	7	97.41±15.96	4.34±2.11	4.63±1.22	133.43±34.08[b]
III	7	95.98±21.65	4.70±1.52	3.87±1.13	83.14±5.87[a]
IV	7	101.21±18.60	4.73±0.99	4.21±0.87	79.71±8.79[a]
V	7	91.98±13.52	4.70±0.88	4.39±1.47	85.57±5.74[a]
p-value		0.897	0.812	0.509	0.001

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

IV. The mean serum of TNF-α in groups III and V tended to be lower when compared to negative and positive controls, but no significant difference was found between groups. The results of serum IL-6 levels showed a relatively similar number for all treatment groups ($p = 0.812$). The mean serum IL-6 tended to be higher in groups III, IV, and V than in the negative and positive controls. Similar results were also found in serum insulin levels. Group III had the lowest insulin levels (3.87±1.13 mIU/L), and the negative control had the highest insulin levels (4.98±1.29 mIU/L).

For fasting blood sugar measurement, it was found that the treatment of brown rice and oyster mushrooms was able to reduce blood sugar levels of rats receiving 50% fructose, indicated by blood sugar levels that were significantly different from positive control ($p = 0.001$) and mean serum levels of GDP were significantly different from negative control. Post-hoc tests found a significant difference between group II and group I ($p = 0.002$), group III ($p = 0.001$), group IV ($p<0.001$), and group V ($p = 0.014$).

There was a very significant effect between the feed treatment on the blood glucose levels of this animal study. The post-hoc test showed that there were differences between treatment groups and positive control groups ($p = 0.001$). This result showed that GBR and oyster mushroom supplementation can reduce the level of blood glucose to a similar level to the negative control group.

After 8 weeks of interventions, glucose levels in blood in the group of metabolic syndrome rats fed with GBR and oyster mushroom supplementation decreased. This was due to the high content of dietary fiber in brown rice and sprouts. Dietary fiber can absorb water and bind glucose, reducing the availability of glucose. A high-fiber diet consists of foods high in complex carbohydrates and fiber which can help to control the sudden rise in blood glucose levels (Ravichanthiran *et al*., 2018). In addition to fiber, anthocyanins in brown rice function as antihyperglycemic. A prior study showed that hyperglycemic rats given anthocyanins from Ayamurasaki sweet potato (100 mg/kg) can reduce blood glucose levels by 16.5% after 30 mins, compared to the control treatment (Suda *et al*., 2003).

Antihyperglycemic effects of anthocyanins are by inhibiting α-glucosidase enzyme activity in producing glucose. The sprout growth in germinated brown rice in diabetic rats can reduce levels of blood glucose compared to normal white rice (Seki *et al*., 2005; Munarko *et al*., 2020) The content of soluble dietary fiber increasing viscosity in the intestinal tract, thereby suppressing diffusion glucose as a result of coated starch molecules and enzymes, which ultimately inhibits amylase activity. Fiber content in GBR sprouts reached 10.76% (Nurhidajah and Nurrahman, 2017). Dietary fiber has the potential to increase the viscosity of the digestive tract, the main reason for the change in the rate of glucose absorption (Maulida and Estiasih, 2014)

In addition, calculations were made for the homeostatic model of insulin resistance (HOMA-IR) and percentage pancreatic beta cell function (HOMA-β). The calculation results can be seen in Table 2. The results showed the highest HOMA-IR value or insulin resistance in the positive control group (normal diet and 50% fructose drink ad libitum) and the lowest in the treatment group I, with the addition of GBR flour supplementation and oyster mushroom ratio of 60:40. As for the value of HOMA-β, the positive control group had the lowest value compared to the other groups. While treatment group II had a HOMA-β value which was almost the same as the normal (negative control) group.

Table 2 shows the HOMA-IR and HOMA-β values between treatment groups. Significant differences were found in the HOMA-IR value, group II or positive control had a higher value than the treatment group (groups III, IV and V). In addition, the treatment group has a value that was not different from group I or negative control. Meanwhile, no significant difference in the HOMA-β value between the treatment groups ($p = 0.06$).

Increased blood glucose (hyperglycemia) and free fatty acids promote the development of oxidative stress,

https://doi.org/10.26656/fr.2017.8(S6).11

Table 2. Mean HOMA-IR and HOMA-β.

	Group					p-value
	I	II	III	IV	V	
HOMA-IR	1.04±0.28[a]	1.50±0.43[b]	0.80±0.27[a]	0.83±0.19[a]	0.95±0.38[a]	0.002
HOMA-β	1.25±0.35	0.76±0.30	0.96±0.26	1.12±0.30	1.05±0.30	0.060

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$).

reactive oxygen species (ROS), and reactive nitrogen species (RNS) (Banerjee and Vats, 2014). This may impair insulin sensitivity and pancreatic beta cell function, worsening the condition of diabetes (Tran *et al*., 2009) During the brown rice germination process, there is an increase in protein breakdown into several amino acids which stimulate insulin secretion, such as alanine (Ala), arginine (Arg), phenylalanine (Phe), isoleucine (Ile), leucine (Leu) and lysine (Lys) (Nurhidajah and Nurrahman, 2017). Insulin deficiency can be caused by a decrease in insulin's ability to reach peripheral tissues (insulin resistance) as well as beta cell dysfunction, which causes the pancreas to produce insufficient insulin to compensate for insulin resistance (Anwer, 2014).

The results showed the highest value of insulin resistance (HOMA-IR) in positive control rats with standard feed and the lowest in the GBR and oyster mushroom formulations 60:40. GBR contains higher fiber than ordinary cracked rice (Kayahara *et al*., 2000). The results of the analysis of dietary fiber in brown rice were 7.98% and increased to 10.76% in the sprouts (Nurhidajah and Nurrahman, 2017). Increased dietary fiber in the diet affects the reduction of insulin resistance (Anderson *et al*., 2009). Insulin resistance reduces insulin-mediated glucose utilization in peripheral tissues (Lee *et al*., 2006). Insulin deficiency resulted in the failure of the insulin receptor substrate (IRS) complex's phosphorylation, decreased GLUT-4 translocation, and glucose oxidation, resulting in glucose being unable to enter cells, a condition known as hyperglycemia (Lee *et al*., 2006). A higher value of HOMA-IR (in the positive control group of mice) caused impaired uptake and use of glucose by body cells, as a result of which blood glucose levels rise. Meanwhile, the greater the HOMA-β value, the better the beta cell strength level (Anwer, 2014; Lee *et al*., 2016).

4. Conclusion

A combination of GBR and oyster mushroom with three different formulations was able to reduce the fasting blood glucose and insulin resistance of rats with metabolic disorders to a similar level to normal or negative control rats. This combination can be served as a supplementation which has the potential as functional food for the management of hyperglycemia and its possible complications.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This study was fully supported by the Implementation of Research and Innovation Consortium Funding Program for Acceleration of Corona Virus Disease 2019 (Covid-19) LPDP and RISTEK/BRIN No. 65/FI/P-KCOVID-19.2B3/IX/2020.

References

Anderson, J.W., Baird, P., Davis Jr, R.H., Ferreri, S., Knudtson, M., Koraym, A., Waters, V. and Williams, C.L. (2009). Health benefits of dietary fiber. *Nutrition Reviews*, 67(4), 188-205. https://doi.org/10.1111/j.1753-4887.2009.00189.x

Anwer, T. (2014). Melatonin ameliorates hyperinsulinemia, glucose intolerance and insulin resistance in STZ-nicotinamide induced type 2 diabetic rats. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(2), 133-136.

Banerjee, M. and Vats, P. (2014). Reactive metabolites and antioxidant gene polymorphisms in type 2 diabetes mellitus. *Indian Journal of Human Genetics*, 20(1), 10-19. https://doi.org/10.4103/0971-6866.132747

de Almeida Brasiel, P.G. (2020). The key role of zinc in elderly immunity: A possible approach in the COVID-19 crisis. *Clinical Nutrition ESPEN*, 38, 65-66. https://doi.org/10.1016/j.clnesp.2020.06.003

Kayahara, H., Tsukahara, K. and Tatai, T. (2000). Flavor, health and nutritional quality of pre-germinated brown rice. In Spanier, A.M., Shahidi, F., Parliment, T.H., Mussinan, C., Ho, C.-T. and Contis, E.T. (Eds.) Food Flavors and Chemistry: Advances of the New Millennium, p. 546-551. UK: The Royal Society of Chemistry. https://doi.org/10.1039/9781847550859-00546

Lee, C.H., Shih, A.Z.L., Woo, Y.C., Fong, C.H.Y., Leung, O.Y., Janus, E., Cheung, B.M.Y. and Lam, K.S.L. (2016). Optimal cut-offs of homeostasis model assessment of insulin resistance (HOMA-IR) to identify dysglycemia and type 2 diabetes mellitus: A 15-year prospective study in Chinese. *PLoS ONE*, 11(9), e0163424. https://doi.org/10.1371/journal.pone.0163424

Lee, J. M., Okumura, M.J., Davis, M.M., Herman, W.H. and Gurney, J.G. (2006). Prevalence and determinants of insulin resistance among U.S. adolescents: A population-based study. *Diabetes Care*, 29(11), 2427-2432. https://doi.org/10.2337/dc06-0709

Maulida, D. and Estiasih, T. (2014). Efek Hipoglikemik Polisakarida Larut Air Umbi Gadung (*Dioscorea hispida*) dan Alginat: Kajian Pustaka. *Jurnal Pangan Dan Agroindustri*, 2(3), 136-140. [In Bahasa Indonesia].

Munarko, H., Sitanggang, A.B., Kusnandar, F. and Budijanto, S. (2020). Beras Coklat Berkecambah (Germinated Brown Rice): Proses Produksi dan Karakteristiknya. *Jurnal Pangan*, 28(3), 239-252. https://doi.org/10.33964/jp.v28i3.436 [In Bahasa Indonesia].

Nurhidajah, N. and Nurrahman, N. (2017). Efek Hipoglikemik Kecambah Beras Merah pada Tikus yang Diinduksi STZ-NA dengan Parameter Kadar Insulin, Indeks HOMA-IR dan HOMA β (Hypoglycemic Effect of Red Rice Germ on Insulin Levels, HOMA-IR, and HOMA β Index of STZ-NA Induced Rats). *Agritech*, 36(4), 433-439. https://doi.org/10.22146/agritech.16767 [In Bahasa Indonesia].

Pranamuda, H., Giarni, R., Pradana, A., Mahsunah, I.S.A. and Dewi, D. (2012). Aplikasi Beta Glukan Sebagai Bahan Berkhasiat Imunomodulator dan Antikanker. Retrieved from https://biofarmaka.ipb.ac.id/biofarmaka/2013/PIRS%202012%20-%20file-KO-TeX_13.pdf [In Bahasa Indonesia].

Qin, C., Zhou, L., Hu, Z., Yang, S., Zhang, S., Chen, M., Yu, H., Tian, D.S. and Wang, W. (2020). Clinical Characteristics and Outcomes of COVID-19 Patients with a History of Stroke in Wuhan, China. *Stroke*, 51(7), 2219-2223. https://doi.org/10.1161/STROKEAHA.120.030365

Ravichanthiran, K., Ma, Z.F., Zhang, H., Cao, Y., Wang, C.W., Muhammad, S., Aglago, E.K., Zhang, Y., Jin, Y. and Pan, B. (2018). Phytochemical profile of brown rice and its nutrigenomic implications. *Antioxidants*, 7(6), 71. https://doi.org/10.3390/antiox7060071

Rothan, H.A. and Byrareddy, S.N. (2020). The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *Journal of Autoimmunity*, 109, 102433. https://doi.org/10.1016/j.jaut.2020.102433

Sekar, A. and Ayustaningwarno, F. (2013). Analisis Kandungan Zat Gizi Makro dan Indeks Glikemik Snack Bar Beras Warna sebagai Makanan Selingan Penderita Nefropati Diabetik. *Journal of Nutrition College*, 2(4), 514-522. https://doi.org/10.14710/jnc.v2i4.3734

Seki, T., Nagase, R., Torimitsu, M., Yanagi, M., Ito, Y., Kise, M., Mizukuchi, A., Fujimura, N., Hayamizu, K. and Ariga, T. (2005). Insoluble fiber is a major constituent responsible for lowering the post-prandial blood glucose concentration in the pre-germinated brown rice. *Biological and Pharmaceutical Bulletin*, 28(8), 1539-1541. https://doi.org/10.1248/bpb.28.1539

Shi, Y., Wang, Y., Shao, C., Huang, J., Gan, J., Huang, X., Bucci, E., Piacentini, M., Ippolito, G. and Melino, G. (2020). COVID-19 infection: the perspectives on immune responses. *Cell Death and Differentiation*, 27(5), 1451-1454. https://doi.org/10.1038/s41418-020-0530-3

Suda, I., Oki, T., Masuda, M., Kobayashi, M., Nishiba, Y. and Furuta, S. (2003). Physiological functionality of purple-fleshed sweet potatoes containing anthocyanins and their utilization in foods. *Japan Agricultural Research Quarterly*, 37(3), 167-173. https://doi.org/10.6090/jarq.37.167

Tay, M.Z., Poh, C.M., Rénia, L., MacAry, P.A. and Ng, L.F.P. (2020). The trinity of COVID-19: immunity, inflammation and intervention. *Nature Reviews Immunology*, 20(6), 363-374. https://doi.org/10.1038/s41577-020-0311-8

Tjokrokusumo, D. (2018). Jamur Tiram (*Pleurotus ostreatus*) Untuk Meningkatkan Ketahanan Pangan Dan Rehabilitasi Lingkungan. *Jurnal Rekayasa Lingkungan*, 4(1), 53-62. https://doi.org/10.29122/jrl.v4i1.1851 [In Bahasa Indonesia].

Tran, L.T., Yuen, V.G. and McNeill, J.H. (2009). The fructose-fed rat: a review on the mechanisms of fructose-induced insulin resistance and hypertension. *Molecular and Cellular Biochemistry,* 332(1), 145-159. https://doi.org/10.1007/S11010-009-0184-4

Weyand, C.M., Yang, Z. and Goronzy, J.J. (2014). T Cell Aging in Rheumatoid Arthritis. *Current Opinion in Rheumatology*, 26(1), 93-100. https://doi.org/10.1097/BOR.0000000000000011

