FABAD J. Pharm. Sci., 46, 2, 129-138, 2021

RESEARCH ARTICLE

Rice Bran Supplement Enhances GSH Levels in Testis and Liver of Carbon Tetrachloride-induced Rats

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Rice Bran Supplement Enhances GSH Levels in Testis and Liver of Carbon Tetrachloride-induced Rats

SUMMARY

In the present study, the potency of rice bran as an antioxidant was examined. Rice bran is a by-product of the rice milling process, despite being a rich nutrient, it has limitations in food application. In this study, we used carbon tetrachloride-induced rats (CCl4) as a model of oxidative stress and examined the effect of extract IPB 3S rice bran supplement (RBS) on testis and liver endogenous antioxidant. The testis and liver were used as the representative organs which prone to exposure to reactive oxygen species (ROS). We used 150 and 300 mg.kg-1 Body weight (BW) of RBS. The Concentration of glutathione (GSH) in both organs was measured. All groups administered by RBS had significantly higher GSH levels compared to the CCl4 group, both in testis and liver. The dose of 300 mg.kg-1 BW RBS had a significantly higher GSH level in testis, while 150 mg.kg-1 VA RBS had a significantly higher GSH level in the liver tissue compared to the control group accordingly. Thus, the rice bran supplement enhances GSH levels in rat's liver and testis which potentially has protective effects.

Key Words: *Rice bran, IPB 3S, antioxidants, glutathione, CCl4, liver, testis.*

Pirinç Kepeği Takviyesi, Karbon Tetraklorürle İndüklenen Sıçanların Testis ve Karaciğerinde GSH Seviyelerini Artırır

ÖZ

Bu çalışmada pirinç kepeğinin antioksidan olarak etkisi incelenmiştir. Pirinç kepeği, pirinç öğütme işleminin bir yan ürünüdür, zengin bir besin olmasına rağmen, gıda uygulamasında sınırlamaları vardır. Bu çalışmada, oksidatif stres modeli olarak karbon tetraklorür indüklenmiş sıçanlar (CCl4) kullanılmış ve IPB 3S pirinç kepeği takviyesinin (PKT) testis ve karaciğer glutatyon (GSH) düzeyleri üzerindeki etkisi incelenmiştir. Testis ve karaciğer, reaktif oksijen türlerine (ROT) maruz kalmaya eğilimli temsili organlar olarak kullanılmıştır. 150 ve 300 mg.kg-1 vücut ağırlığı (VA) PKT kulanılmıştır. Her iki organda GSH konsantrasyonu belirlenmiştir. PKT uygulanan tüm gruplarda, hem testis hem de karaciğerde CCl4 grubuna kıyasla anlamlı derecede daha yüksek GSH düzeyleri görülmüştür. 300 mg.kg-1 VA PKT dozda testiste GSH düzeyi kontrole göre anlamlı derecede yüksek iken 150 mg.kg-1 VA PKT dozunda karaciğer dokusunda GSH düzeyi kontrol grubuna göre anlamlı derecede yüksek bulunmuştur. Dolayısıyla, pirinç kepeği takviyesi, potansiyel olarak koruyucu etkileri olan GSH seviyelerini sıçan karaciğer ve testis dokusunda artırmaktadır.

Anahtar Kelimeler: *Pirinç kepeği, IPB 3S, antioksidanlar, glutatyon, CCl4, karaciğer, testis.*

Received: 27.07.2020
Revised: 15.02.2021
Accepted: 27.03.2021

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INTRODUCTION

Reactive oxygen species (ROS) have broad deleterious effects on the human body and play an important role in the aging process, cardiovascular disease, cancer, cataracts, immune system disorders, brain dysfunction, and infertility. ROS reacts with proteins, lipids, carbohydrates, and nucleic acids cause oxidative damage which leads to impair cell function and apoptosis (Bender, 2009). To protect cells from oxidative damage, the human body produces defense mechanisms including enzymatic antioxidants (catalase, superoxide dismutase, and glutathione peroxidase) and non-enzymatic antioxidants (glutathione). The antioxidant can also be gained from food, such as fruits, vegetables, and rice (Carlsen, 2010; Lobo, 2010; Mottillo, 2010). One of the important endogenous antioxidants in our body is glutathione. Glutathione is a low-molecular-mass, thiol-containing tripeptide, which has two forms, glutamic acid-cysteine-glycine (GSH) in its reduced form and GSSG in its oxidized form. The GSH/GSSG is considered as an important player in the cellular redox system since it reacts directly with the free radicals and donates an electron to remove the hydrogen peroxide (H_2O_2) to prevent oxidative stress damage within the cell (Kohen & Nyska, 2002; Szymonik-Lesiuk, 2003; Bender, 2009). Therefore, any impairment of GSH in living organisms may lead to tissue disorder or injury.

Testis and liver are organs that prone to have ROS exposure. The increased generation of free radicals such as ROS plays an important role in testicular damage. The sperms are generated within the testes and are known as the spermatogenesis process. This process is a very active replication process and produces around 1000 sperm per second (Turner & Lysiak, 2008). The high cell division rate in spermatogenesis causes an increase in mitochondrial oxygen consumption by the germinates epithelium (Turner & Lysiak, 2008; Ourique, 2016). It makes the testis as an organ that susceptible to oxidative stress resulting in infertility. Nowadays, the increase of environmental exposure to pollutants, such as pollution, metal exposure, pesticides, and unhealthy lifestyles such as smoking and alcohol consumption, leads to high free radical production in all body tissues, including the testis (Turner & Lysiak, 2008). Thus, increased the testis risk for oxidative stress damage.

As the main metabolic organ, the liver is also prone to exposure to ROS. ROS is known to be primarily produced in the mitochondria and the endoplasmic reticulum of hepatocytes through cytochrome P450 enzymes. Thus, the cellular components of hepatocytes such as lipids, proteins, and DNA are susceptible and primarily affected by ROS. Under normal conditions, the cells in our body are armored with special molecular mechanisms that manage the oxidative stress level and maintain the homeostasis between pro-oxidant and anti-oxidant molecules. The liver is therefore armored with a special defense mechanism to eliminate the ROS. Increased levels of ROS will cause the release of nuclear factor E2-related factor 2 (Nrf2) and it will translocate to the nucleus to promote the transcription of cytoprotective genes such as GSH (Cichoz-Lach & Michalak, 2014). GSH is a major endogenous antioxidant in hepatocytes. The homeostasis of GSH/GSSG plays an important role in maintaining the functions of cellular proteins and enzymes involved in the pathway of cell death and survival (Yuan & Kaplowitz, 2009). The GSH is therefore crucial for defense mechanisms against ROS in hepatocytes.

Studies have reported that the response of endogenous antioxidants is varied in different tissues during oxidative stress. Moreover, both enzymatic and non-enzymatic antioxidant systems are limited, therefore in prolonged pathologic conditions, the damages can be irreversible. GSH is known to play a crucial role in the liver, while in testis it is also crucial for the maturation and storage of the spermatozoa (Kaneko, 2002; Szymonik-Lesiuk, 2003; Turner & Lysiak, 2008; Yuan & Kaplowitz, 2009; Cichoz-Lach & Michalak, 2014). To create the condition which has oxidative stress in several tissues/ organs, in this study we used carbon tetrachloride (CCl_4) induction in the

animal model. Moreover, CCl_4 induction is proven to have other oxidative damage effects despite the hepatotoxicity known effect (Szymonik-Lesiuk, 2003; Dalton, 2009).

Rice bran is a by-product of the rice milling process responsible for 10% of rice constitutes. In Indonesia, rice as staple food continues to increase in consumption. To improve rice productivity and accelerate to food self-sufficiency in Indonesia, the IPB 3S as a new variety of rice was introduced by Bogor Agriculture University, in Indonesia (Aswidinnoor, 2017; Anisa Tika Padar Wati, 2019). The increase in rice-producing activities will also cause an increase in its side products including rice bran. Studies have reported that rice bran is rich in B and E vitamins, and also *γ-oryzanol* which is beneficial to protect cells from oxidative stress (Xu, 2001; Arab, 2011; Islam, 2014). The previous study by Spiazzi (2013) showed that *γ-oryzanol* prevents oxidative damage in mice testis (Spiazzi, 2013). While the study by Al-Okbi (2014), found that rice bran oil showed a hepato-protective effect against non-alcoholic steatohepatitis (NASH) in rat liver (Al-Okbi, 2014). The potential advantages of rice bran which is rich in nutrients and vitamins have been shown in researches, however, most of its usage is as animal feed or even discarded as waste materials. The utilization of rice bran as an antioxidant supplement is still also very limited. Considering the beneficial effect of rice bran, in this study we aimed to evaluate the potential antioxidant effect of IPB 3S rice bran supplement in the testis and liver of CCl_4-induced rats through measurement of GSH level.

MATERIALS AND METHODS

Experimental animals and design

This study was held in the Department of Biochemistry and Molecular Biology, Faculty of Medicine Universitas Indonesia. All experiments in this study were performed by following the guidelines for animal research from the National Institutes of Health and were approved by the Ethical Committee Faculty of Medicine Universitas Indonesia (No.514/UN2.F1/ETIK/2016). We used *Sprague-Dawley* male rats aged 6-8 weeks from PT Bio Farma, Bandung Indonesia. The rat's body weights are close to each other with an average (206.67 $\pm$ 11.14) gram. The rats were housed in cages with a 12-h light/dark cycle and were had free access to the food and drinking water throughout the experiment. They were acclimatized for 1-week before the experiment. We used 24 rats which randomly divided into 6 groups including control; induction of CCl_4 0.55 $mg.kg^{-1}$ body weight (BW); 150 $mg.kg^{-1}$ BW rice bran brain supplement (RBS); 150 $mg.kg^{-1}$ BW RBS + CCl_4; 300 $mg.kg^{-1}$ BW RBS; and 300 $mg.kg^{-1}$ BW RBS + CCl_4. The rationale of the doses of CCl_4 and RBS was based on the previous study (Oktavinda & Dwirini Retno Gunarti, 2021).

Rice bran extract preparation and CCl_4 induction

IPB 3S rice bran was sifted and grind. Then, 100 ml NaCl 0.9% was added to 10 g of bran. The animals were fed with the rice bran once a day following the instructed dose for each group, and performed in a week. On the last day of the intervention, 0.55 $mg.kg^{-1}$ BW of CCl_4 was induced once in the determined groups. Rats were sacrificed at the end of the treatment series. The testis and liver were taken as samples and placed on the test tube, stored at -80 °C before use.

GSH measurement

First, we prepared for tissue homogenates. Small portions of tissues were excised from liver and testis organs as much as 100 mg; they were then homogenated by adding 1 ml of phosphate buffer saline (PBS) and crushed using a manual homogenizer, the process was performed on ice. The solutions were then centrifuged at 2096 x *g* for 10 minutes. The supernatants were taken as samples.

GSH levels were measured using the modification of *Ellman's method* as previously described (Rahman, 2007; Nurrochmad, 2010). We prepared for 3 groups of tubes, consisted of blank, standard, and test tubes, each was duple. The blank tube was fulfilled by distilled water 50 µl, while the standard tubes were fulfilled with 2 mg/ml of glutathione in PBS and divided into 1 µl, 2 µl, 4 µl, 5 µl, and 10 µl. While the test tube was fulfilled by 50 µl sample. All tubes were added with 200 µl trichloroacetic acid (TCA) 5%. Then, they were centrifuged for 10 minutes at the speed of 1027

x *g*. The supernatant was taken and added 25 µl of 5, 5'-dithio bis2-nitrobenzoic acid (DTNB/ Ellman's reagent) 0.1M for all of the tubes. All of the test tubes were finally made up of a volume of 2 ml by added PBS pH 8.0. They all then incubated in the darkroom for 60 minutes, then the absorbance was read using a spectrophotometer (λ = 412 nm).

Statistical Analysis

We used Microsoft Excel to set the linear equation with the determinant coefficient for measuring the level of GSH. Data analysis was done by using SPSS version 20. Shapiro-Wilk test was used to determine the normality data, while Levene's test was used to determine the homogeneity of data. The mean difference of GSH levels in each group was analyzed using the one-way ANOVA test, *p*-value <0.05 was considered statistically significant.

RESULTS AND DISCUSSIONS

The effect of IPB 3S rice bran supplement on GSH concentrations in testis

To calculate the GSH level in testis, the GSH standard curve was needed. GSH standard curve was obtained by measuring the absorbance values at various standardized GSH concentrations. The x-axis showed the substrate concentration and the y-axis showed the absorbance results. The equation was y = 0.0349x + 0.0056, which was obtained from the GSH standard curve (Figure 1). With this equation, using the formula of x = (y-0.0056) / 0.0349 we then calculate the value of GSH concentration.

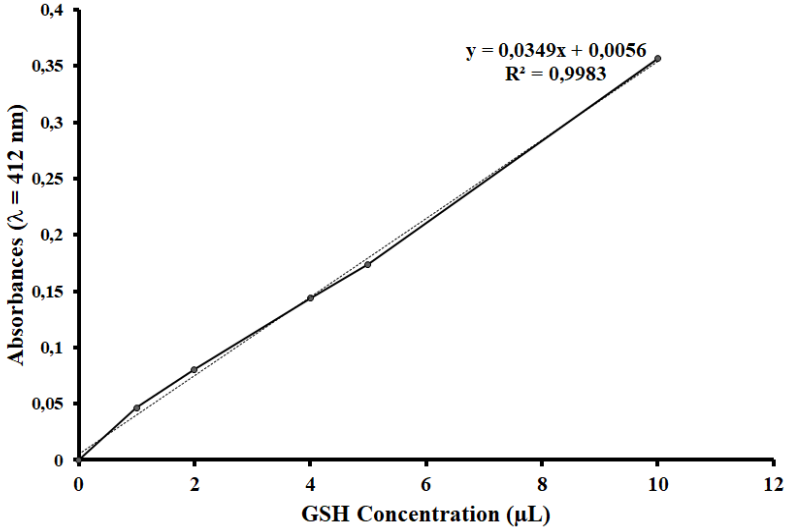

Figure 1. Standard curve of GSH concentration.

The mean of GSH level in all groups administered by RBS, either with or without the induction of CCl_4, tend to have a higher level than the group induced by CCl_4 only. The dose of RBS 300 mg.kg^{-1} BW significantly has higher GSH level (4.41 ± 0.53 pmol. mg^{-1} tissue) compared to control (3.28 ± 0.12 pmol. mg^{-1} tissue) group and CCl_4 only group ((3.01 ± 0.49 pmol.mg^{-1} tissue) (p <0.05). While the GSH level in the group administered by a dose of 150 mg.kg^{-1} BW of RBS (3.86 ± 0.34 pmol.mg^{-1} tissue) was not significantly different to control (p >0.05) (Figure 2).

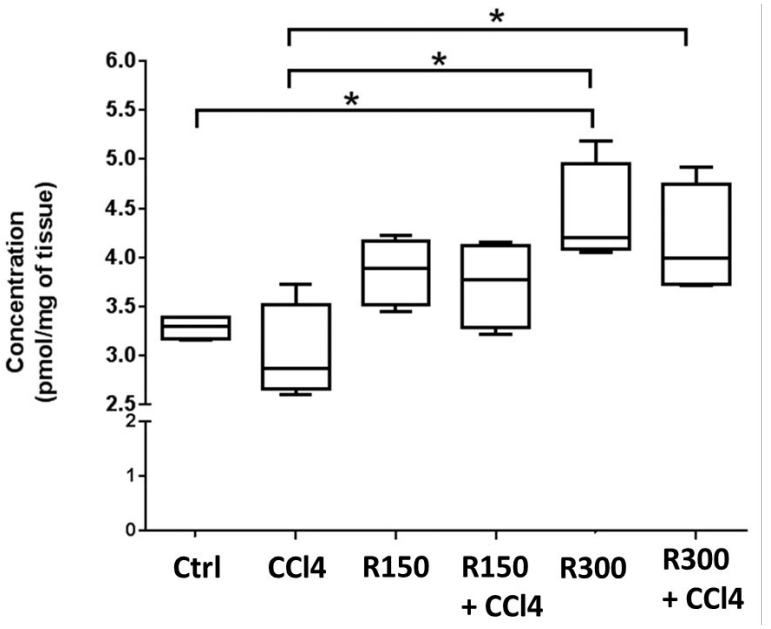

Figure 2. GSH concentration in testis tissue between each groups.
Ctrl – control, R– rice bran supplement, CCl4 – carbon tetrachloride.
The values denoted by an asterisk (*) is differ significantly at $p \leq 0.05$.

The CCl_4 is known to cause testicular toxicity through biotransformation by the cytochrome P-450 system to form trichloromethyl radical (CCl_3) which then turns into trichloromethyl peroxyl radical (CCl_3O_2) (Szymonik-Lesiuk, 2003). The CCl_4 metabolites reacted with unsaturated fatty acids forming covalent bonds with lipids and proteins resulting in the formation of lipid peroxidase and destruction of cell membranes that caused injury to the testis tissue (Szymonik-Lesiuk, 2003). In this study, we have demonstrated that the induction of CCl_4 in rats caused low GSH levels in testis tissue. Our study was in line with the previous study by Al-Olayan (2014) Which demonstrated that CCl_4 induction caused a significant reduction in GSH levels of testis compared to control. In that study, the CCl_4 was given for a long time (every week for 10 weeks) with a higher dose (2 $ml.kg^{-1}$). Moreover, the CCl_4 induction showed its deleterious effect on testis tissue as it causes the degeneration of seminiferous tubules and germ cells, and some parts of the interstitial tissues were filled in with fibroblasts and inflammatory cells.

Spermatozoa are very susceptible to oxidative damage due to high levels of unsaturated lipids in the plasma membrane (Spiazzi, 2013). A study by Xu (2001) showed that γ-oryzanol was the main antioxidant compound in rice bran, followed by vitamin E The γ-oryzanol was able to prevent lipid peroxidase in the testis of rat-induced Cadmium intoxication. Moreover, the γ-oryzanol ingredients also demonstrated to increase superoxide dismutase (SOD) and glutathione S-transferases (GST) activity to fight the oxidative stress (Spiazzi, 2013). In addition, the study conducted by Adriani (2012) reported that adding the doses of rice bran to Swiss Webster mice improved the quality of spermatogenesis by increasing the diameter of the seminiferous tubules.

Other studies also support that testis is the organ that is susceptible enough against oxidative stress-in-

duced valproic acid. The administration of vitamin E on rats treated with valproic acids showed to prevent valproic acid-induced oxidative damage in testes and epididymis which rescues sperm motility and enhances antioxidant defenses compared to the control group. The antioxidant of vitamin E provided a protective effect against lipid peroxidase and increased enzymatic antioxidants such as SOD, CAT, GSH-Px, and GST in rat testis (Ognjanović, 2010; N. Wang, 2012; Ourique, 2016). Our study was in line with those previous studies, where administration of RBS with a dose of 300 mg.kg^{-1} BW showed to promote higher GSH levels in testis more than the dose of 150 mg.kg^{-1} BW. Moreover, the addition of CCl_4 in conjunction with 300 mg.kg^{-1} BW RBS showed to have significantly higher GSH levels in testis tissue compared to CCl_4 only. This result supports the preventive effect of RBS to fight the oxidative stress induced by CCl_4.

The effect of IPB 3S rice bran supplement on GSH concentrations in liver

Using a similar standard curve as in testis, we calculated the GSH concentration. The treatment group of 150 mg.kg^{-1} BW RBS significantly has the highest GSH levels (3.75 $\pm$ 0.10 pmol.mg^{-1} tissue) compared to the other groups (p<0.05). The CCl_4 group has significantly the lowest GSH level (2.73 $\pm$ 0.08 pmol.mg^{-1} tissue) compared to the other groups (p<0.05). Moreover, the level of GSH in the treatment groups of 150 mg.kg^{-1} BW RBS, either with or without the induction of CCl_4, were significantly higher than control (3.17 $\pm$ 0.10 pmol.mg^{-1} tissue) group (p = 0.001 and p = 0.046) and the CCl_4 group (p <0.001). Meanwhile, the GSH levels in the group treated by a higher dose of RBS (300 mg.kg^{-1} BW) show comparable levels (3.41 $\pm$ 0.16 pmol. mg^{-1} tissue) to the control group (p >0.05) (Figure 3).

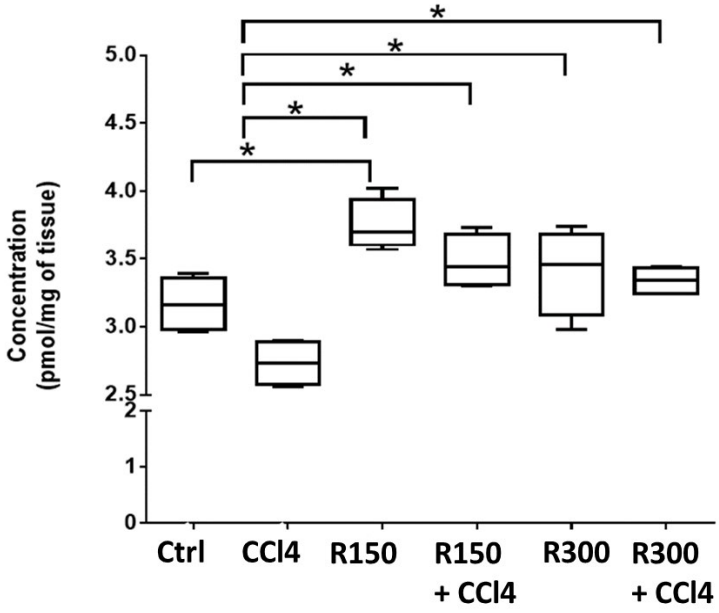

Figure 3. GSH concentration in liver tissue between each groups.

Ctrl – control, R– rice bran supplement, CCl4 – carbon tetrachloride.
The values denoted by an asterisk (*) is differ significantly at $p \leq 0.05$.

The liver injury induced by hepatotoxic agents such as paracetamol and CCl_4 is known to be correlated with low tissue levels of GSH (Leeuwenburgh & Ji, 1995). The toxicity of CCl_4 was the result of CCl_4 bio-activation producing trichloromethyl free radical by CYP450 in the hepatic microsome. It led to lipid peroxidation in the membrane cell causing the damage of hepatocytes (Szymonik-Lesiuk, 2003; Lobo, 2010). Therefore, in our study, we did not determine lipid peroxidation. The induction of CCl_4 showed a significant increase in hepatic enzymes (AST, ALT, and ALP) and bilirubin levels, indicating the damage of liver organ structure (Adewale, 2014; Singh, 2014; Li, 2016). Moreover, the study by Li, also proved that fibrosis of liver organs was developed in the subjects induced by CCl_4 based on histology examination of the liver (Li, 2016). Our results also showed that the CCl_4 group has the lowest GSH levels in liver tissue compared to the control group ($p<0.05$), which supported the oxidative stress animal model by induction of CCl_4.

As mentioned before, the γ-oryzanol and vitamin E ingredients within RBS are thought as the main function of the antioxidant property of RBS. Sengupta (2014) have shown that rice bran (oil) administration increased GSH and glutathione peroxidase levels in Wistar rats induced by sodium arsenite. Wang (2014) also found that the aqueous enzymatic extract of rice bran (AEERB) in the dose of 750 $mg.kg^{-1}$ significantly increased the level of glutathione peroxide compared to the subjects administered by hyperlipidemic diet. Our study also in line with those previous studies, the RBS administration shows a significant effect in promoting higher GSH levels compared to the control and CCl_4 groups in both doses of 150 $mg.kg^{-1}$ and 300 $mg.kg^{-1}$ BW. However, the prominent benefit was shown by the dose of RBS 150 $mg.kg^{-1}$ BW. Under the normal or untreated condition, the dose of 300 $mg.kg^{-1}$ BW RBS significantly promotes the increase of GSH levels in testicles, while in the liver it only promotes a slight increase of GSH levels. These differences may due to tissue-specific reactions to the RBS, and the facts that each tissue or organ may have different levels of GSH as shown by several studies (Hariharakrishnan, 2009; Wang, 2014). Moreover, the addition of CCl_4 in conjunction with RBS still showed significantly higher levels of GSH compared to CCl_4 only group. This indicates the benefit of RBS for preventive action against oxidative stress. The increased GSH level is known to have a crucial role in the protection of hepatocytes (Yuan & Kaplowitz, 2009; Y. X. Wang, 2014).

In the present study, the GSH levels in both testis and liver were higher with the administration of RBS in both control and CCl_4 treated groups. GSH is one of the first-line endogenous antioxidant defense systems. GSH protects the cells from oxidative stress by converting the ROS to less reactive species through direct scavenging of superoxide radicals and hydrogen peroxide (Sengupta, 2014). The restoration of GSH levels in both testis and liver tissues with RBS may due to an increase of intracellular concentration of the glutathione reductase (GR). The GR will then regenerate GSH from GSSG with NADPH as a source of hydrogen (Sengupta, 2014). The main possible reason for the improvement of GSH levels is that γ-oryzanol as the main antioxidant component of rice bran promotes the intracellular SH-group-containing compounds including glutathione (Spiazzi, 2013). Moreover, a study by (Rungratanawanich, 2018) has demonstrated that the underlying antioxidant mechanism of γ-oryzanol is in part through the initiation of Nrf2 translocation followed by an increase of the expression of Nrf2 dependent genes at both mRNA and protein levels. Another proposed mechanism action of γ-oryzanol is suggested by its capacity as ROS scavengers and prevent lipid peroxidation, leads to a complex network of interactions, and heightens in organ-cell specific responses (Minatel, 2016). Further studies may require to confirm these beneficial effects in histopathological changes and the expression of Nrf2 in both testis and liver tissues.

CONCLUSIONS

Our study suggested the antioxidant effects of rice bran supplement of IPB 3S (RBS) against the oxidative stress-induced with CCl_4 in testis and liver of male rats. The depletion of GSH levels in the CCl_4 group was reversed back to a similar level as in control by co-administration of RBS. Moreover, the eminent effect of RBS alone on male rats was the enhanced level of GSH in both testis and liver tissues. Further studies are required to explore the biochemical and histological parameters to support the protective effect of IPB 3S bran supplement in organs to prevent oxidative damage.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTION STATEMENT

Conception and design (Gunarti, D.R.), collection and/or assembly of the data (Nasser, M. K., Ikram, T. A. Z., Pribawa, R. N., Gunarti, D.R.), literature research (Sukmawati, D., Suryandari, D. A.), preparing the study text (Sukmawati, D., Nasser, M. K., Ikram, T. A. Z., Pribawa, R. N.), statistical analysis and interpretation of the data (Nasser, M. K., Ikram, T. A. Z., Pribawa, R. N.), reviewing the text (Sukmawati, D., Gunarti, D. R., Suryandari, D. A.), funding/grants application (Sukmawati, D., Gunarti, D. R., Suryandari, D. A.)

ACKNOWLEDGMENTS

This study was supported by a research grant (Hibah PITTA B 2019, No. NKB-0530/UN2.R3.1/HKP.05.00/2019) funded by the Directorate of Research and Community Engagement, Universitas Indonesia. Authors convey their appreciation to laboratory staff in the Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia for technical assistance during the experiments.

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