The potential effect of temulawak (Curcuma xanthorrhiza Roxb.) and garlic (Allium sativum L.) as a radioprotective agent against 6 Gy total body irradiation in rats

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Abstract – The main impact of gamma radiation on a biological system is the formation of Reactive Oxygen Species (ROS) and free radicals. The study aimed to explore the potential effect of temulawak (Curcuma xanthorrhiza Roxb.) and garlic (Allium sativum L.) against 6 Gy total body irradiation (TBI) in rats by observing malondialdehyde (MDA), glutathione (GSH) and comet assay. Twenty male rats were divided into five groups: control, 6 Gy, temulawak extract + 6 Gy, garlic extract + 6 Gy, and n-acetyl cysteine (NAC) + 6 Gy. MDA and GSH were measured on liver and spleen tissue homogenates, while comet assay was on lymphocyte cells. Gamma irradiation at 6 Gy significantly increased the MDA level and comet assay compared to the control group, while the GSH level decreased (p < 0.05). Temulawak extract significantly reduced MDA levels and comet assay compared to the 6 Gy group while increasing GSH levels in the liver. Garlic extract significantly drops comet assay while increasing GSH levels in the liver. NAC decreases MDA levels in the liver and comet assay while increasing GSH levels in the spleen. It could be concluded that temulawak extract has a better radioprotective agent than garlic extract and is almost identical to NAC as a standard antioxidant.

Keywords: temulawak / garlic / radioprotective agent / free radical / antioxidant

1 Introduction

Gamma radiation has been comprehensively explored for its application in the medical field (Liju et al., 2020). The effects of exposure to low doses of gamma radiation on medical radiation workers concern health researchers. Medical radiation workers include hospitals, clinics, and private employees that use ionizing radiation in their services. Biological damage to normal cells is a significant occupational health problem for radiation workers (Gao et al., 2020). Nucleic acids, proteins, and lipids are the main biomacromolecules affected by gamma radiation (Mercantepe et al., 1977; Jit et al., 2022).

Gamma radiation on cells will produce free radicals interacting with polyunsaturated fatty acids (PUFAs), thus forming lipid peroxide compounds and reactive oxygen species (ROS). The primary product of lipid peroxidation is the formation of malondialdehyde (MDA) compounds. It indicates the presence of free radicals in cells (Abou El-Eneen et al., 2020). El-Desouky et al. (2017) revealed that single doses of 5 Gy and 10 Gy gamma radiation could induce lipid peroxidation in rats. At the DNA level, free radicals can initiate DNA single-strand breaks (SSBs) and double-strand breaks (DSBs), causing mutations and disrupting genome integrity. The comet assay is one of the most sensitive and rapid methods for detecting the spontaneous level of DNA damage at the individual level (Nair and Menon, 2013).

Pointedly, the body has natural endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and glutathione peroxidase (GPx), which protect cells from the risk of free radicals. Excessive free radicals will reduce the availability of endogenous anti-oxidants, causing oxidative stress (Abou El-Eneen et al., 2020).

A radioprotective agent is a substance/drug used to protect healthy cells from the toxic effects of IR selectively.
Radioprotectors can protect against radiation because of their ability to bind radicals resulting from the radiolysis of water. Furthermore, antioxidants can act as radioprotectors due to the similarities between oxidative stress and radiation injury. Therefore, an ideal radioprotector should offer significant protection against the long-term effects of radiation exposure (Caro et al., 2012; Obrador et al., 2020). At this time, the use of natural compounds to protect human health is increasing. The choice of radioprotector is more emphasized in plant products (Obrador et al., 2020; Javadi et al., 2022; Jit et al., 2022).

Several studies have proven that many traditional plants have antioxidant capacity and act as radical scavengers. Temulawak (Curcuma xanthorrhiza Roxb.) is a plant from Indonesia that is useful for medicine. The most active components are curcumin, which results from the secondary metabolism of temulawak and is considered to have a strong antioxidant capacity (Atun et al., 2020; Rosidi, 2020; Shabeeb et al., 2020). Garlic (Allium sativum L.) is scientifically proven to have antioxidant capacities. Its antioxidant activity is in water-soluble organosulfur compounds, such as S-alliycysteine and S-allylmercaptocysteine. (Caro et al., 2012; Bertrand et al., 2016). N-acetylcysteine (NAC) is a derivative of cysteine, a thiol-reducing agent found in vegetables such as garlic, onions, and peppers. NAC is commonly used as a standard antioxidant because of its ability to inhibit oxidative stress and suppress DNA damage (Mercantepe et al., 1977; Kilicikiz et al., 2008).

The present study focused on exploring the ability of temulawak and garlic extract to suppress oxidative damage caused by 6 Gy TBI in Wistar rats.

### 2 Materials and methods

#### 2.1 Materials and extract preparation

Male Wistar rats, 8–12 weeks old, weighing 180–220 g, were used in the present study. The animals were purchased from iRATco Veterinary Laboratory Service, Bogor, Indonesia. The animals were housed and acclimatized in temperature conditions (21–24 °C) and optimum lighting (12 h dark/light cycle) in the Integrated Animal Laboratory, National Research and Innovation Agency (BRIN), Indonesia. The animals were fed a pelleted rodent diet (iRATco), water ad libitum, and weighed regularly once a week.

Temulawak and garlic were obtained from Indonesian Farmers Store, while NAC was purchased from Sigma-Aldrich (product number A7250).

Temulawak extract was made at the Indonesian Medicinal and Aromatic Crops Research Institute (IMACRI) using the phytochemical method. The dose of temulawak extract was used 100 mg per kilogram body weight (mg/kg BW) (Liju et al., 2020). At the same time, the garlic extract was done by following the previously published method (Kisnanto et al., 2020).

#### 2.2 Animal treatment, irradiation, and sample collection

Twenty male Wistar rats were classified into five groups (N = 4 each): control (non-irradiation), 6 Gy, temulawak extract + 6 Gy, garlic extract + 6 Gy, and NAC + 6 Gy. The extracts were administered gavage orally for seven consecutive days.

Six Gy with a dose rate of 1 Gy/min TBI was given on the eighth day using a gamma ray cobalt-60 IRPASENA device at the Research Center for Radiation Process Technology, BRIN, Indonesia. After 24 h post-irradiation, all rats were anaesthetized and euthanized with ketamine-xylazine (75–100 mg/kgBW) intraperitoneally. Surgery was performed by opening the thoracic cage, and the blood was removed directly using a 10 mL syringe. The blood was placed into the EDTA tube for lymphocyte isolation. After blood collection, the liver and the spleen were immediately removed and preserved at −80 °C for further use.

#### 2.3 Lymphocyte isolation and tissue homogenate

Hasan Basri et al. (2017) determined the lymphocyte isolation procedure with slight modification. Anticoagulated blood samples were mixed with 3 mL of Phosphate Buffered Saline (PBS) pH 7.6 in a centrifugation tube. The blood cells were carefully mixed through the walls of the tube containing 3 mL of lymphocyte-separating medium (Histopaque 1077), followed by centrifugation for 30 min at 1500 rpm. The layer of lymphocyte cells looks slightly greyish white, located between the blood plasma and Histopaque, and then transferred to a centrifuge tube containing 5 mL of PBS (pH 7.6), followed by centrifugation for 15 min at 1000 rpm. The lymphocyte cells were washed three times with PBS (pH 7.6). The supernatants were removed, while the pellets were resuspended by adding RPMI-1640I medium (Sigma-Aldrich), stored at −80 °C.

The liver and the spleen tissue (each 100 mg) were dissolved in 0.5 mL of 0.01 MPBS (pH 7.4) and then homogenized using a homogenizer machine. The mixture was centrifuged for 10 min at 3500 rpm. The supernatant was taken and stored at −80 °C.

#### 2.4 Measurement of MDA level

The MDA levels were measured based on the method of Wills (1971) with some modifications. The MDA level was assayed using spectrophotometry (Genesys 20), and absorbance was read at a wavelength of 530 nm. The MDA level was expressed in nmol/mg tissue.

#### 2.5 Measurement of total protein concentration

Determination of the total protein concentration in the liver and spleen tissue was based on the method by Mercantepe et al. (1977) with some modifications.

#### 2.6 Measurement of GSH level

The GSH concentration was assayed based on the method of Ellman (1959) with modifications. The GSH concentration was assayed using spectrophotometry (Genesys 20), and absorbance was read at a wavelength of 412 nm. The GSH
level was expressed in total protein concentration (µg/mg protein).

2.7 The alkaline comet assay

The comet assay procedure was done according to the method of Darlina et al. (2022) with minor modifications.

2.8 Statistical analysis

The data were analyzed using IBM SPSS Statistics 24.0 software. Data are presented as mean ± SD and considered statistically significant at $p < 0.05$. Statistical analyses were conducted using one-way analysis of variance (ANOVA) with LSD post hoc tests where appropriate.

3 Results

3.1 MDA level

The effect of gamma irradiation and radioprotectant agents on MDA levels can be seen in Figure 1. The results of the current study explained that levels of MDA in the liver and spleen tissue in the 6 Gy group liven up significantly compared to the control group ($p = 0.004; p = 0.02$). Meanwhile, radioprotectant agents (temulawak, garlic, and NAC) were administered prior to gamma irradiation and showed slightly different data on MDA levels in the liver and spleen.

Temulawak extract significantly declined MDA levels in the liver and spleen compared to the 6 Gy group ($p = 0.003; p = 0.008$). Garlic extract could not significantly drop MDA levels in the liver and spleen ($p = 0.069; p = 0.064$). NAC could only significantly reduce MDA levels in the liver ($p = 0.012$), whereas, in the spleen, it was not significant ($p = 0.642$).

3.2 GSH level

Figure 2 describes the effect of gamma irradiation and radioprotectant agents on GSH levels. The lowest GSH levels were observed in the 6 Gy group and significantly differed from the control group in the liver tissue ($p = 0.015$) and spleen tissue ($p = 0.003$).

In the liver tissue, temulawak and garlic extracts elevate GSH levels significantly compared to the 6 Gy group ($p = 0.021; p = 0.044$). NAC could also liven up GSH levels, but not significantly different. In contrast, only NAC significantly increased GSH levels in the spleen tissue compared to the 6 Gy group ($p = 0.010$). Meanwhile, temu-lawak and garlic extracts also increased GSH levels but were not significantly different ($p = 0.210; p = 0.519$).

3.3 The alkaline comet assay

The alkaline comet assay was carried out to examine the extent of spontaneous DNA damage in rat blood lymphocyte cells with four parameters assay, namely Tail Length (TL), Tail

Fig. 1. Effect of radioprotectant agent supplementation on MDA levels in the liver (A) and spleen (B) tissue homogenates of Wistar rats exposed to 6 Gy TBI.

*Significant different compare to the 6 Gy group ($p < 0.05$, LSD post hoc test); ns = not significant.
DNA (T. DNA), Tail Moment (TM), and Olive Tail Moment (OTM). The results concluded that spontaneous DNA damage for all parameters in the 6 Gy group was significantly higher than in the control group. However, the increased comet parameters were significantly weakened in the presence of a radioprotectant agent on almost all parameters, as evident from Figure 3. In addition, different results were obtained for the temulawak extract, which did not significantly differ in the T.DNA parameter ($p = 0.091$).

DNA damage due to IR has been demonstrated by the microscopic appearance of the most extended comet tail in the 6 Gy group. However, the comet tail length was significantly lessened after supplementing with temulawak, garlic, and NAC extracts ($p < 0.01$) (Fig. 4).

### 4 Discussion

The current study evaluated the potential of temulawak, garlic extract, and NAC as radioprotectant agents against IR exposure in Wistar rats. The assessment was performed on the corrective of lipid peroxidation damage, an enhancement in the antioxidant status of GSH, and the ability to protect against cellular DNA damage.

Six Gy TBI causes tissue damage in the liver and spleen, as demonstrated by a significant increase in MDA levels compared to the control group. It is possible because of free radical onset on PUFAs components in lipid membranes. In addition, radiation-induced oxidative damage causes changes in the fluidity and permeability of the lipid membranes (Kilciksiz et al., 2008). We also noted degradation in MDA levels after administering a radioprotectant agent prior to irradiation. Temulawak extract and NAC supplementation in the liver tissue significantly decreased MDA levels compared to the 6 Gy group, but not with garlic extract.

However, in the spleen tissue, only temulawak extract had a significant effect on reducing MDA levels. Based on a study by Rosidi (2020), about 61–67% of the active antioxidant compounds in temulawak are curcumin. Curcumin is a potent antioxidant that can prevent free radical chain reactions, lowering lipid peroxidation damage and upgrading endogenous antioxidant performance (Ozcelik et al., 2018). Our study is in line with the study of Ozcelik et al. (2018), who reported that the administration of curcumin could significantly reduce MDA levels in the kidneys and brains of rats by 9 Gy gamma radiation-induced. Various investigators also reported the substantial benefit of curcumin in suppressing the breakdown of lipid peroxidation in hepato-testicular (Ammar, 2016), skin tissue (Shabeeb et al., 2020), and bone marrow (Bagheri et al., 2018) in rats.

Similar results have been noted by Mansour et al. (2008), who stated that the treatment of NAC (1 g/KgBW) for seven consecutive days prior to 6 Gy of gamma radiation-induced could reduce MDA levels return to near average values in rat liver tissue. In contrast, garlic extract did not give a significant difference to the decrease in MDA levels in all tissues.
Fig. 3. Effect of radioprotectant agent supplementation on spontaneous DNA damage in the lymphocyte cell of Wistar rats exposed to 6 Gy TBI based on TL (A), T.DNA (B), TM (C), and OTM (D) parameters.

*Significant different compare to the 6 Gy group \((p < 0.05, \text{LSD post hoc test})\); ns = not significant.
We assume that although garlic contains potent antioxidant active substances, insufficient to counteract the quantity of ROS.

Different from several previous studies, garlic plays a role in inhibiting the velocity of lipid peroxidation. Batcioglu et al. (2012) observed that the supplementation of garlic extract (500 mg/mL) for 30 consecutive days significantly declined MDA levels in liver and kidney tissues of rats by 20 Gy gamma radiation-induced. Meanwhile, Bertrand et al. (2016) reported that Age Garlic Extract (AGE) could inhibit lipid membrane damage in the liver, spleen, thymus, kidney, and testes of Wistar rats by 4.5 Gy radiation-induced.

GSH with GPx can catalyze reducing lipid hydroperoxides to OH* and H2O. GSH plays a primary role in the endogenous defence system against oxidative damage through its radical scavenging and repair features of protein and nonprotein macromolecules (Ozcelik et al., 2018). Our study reported that 6 Gy TBI causes tissue damage in the liver and spleen, as demonstrated by a significant drop in GSH levels compared to the control group. IR exposure inflicts the depletion of GSH levels caused by excessive liver and spleen damage resulting in oxidative stress conditions. IR exposure leads to protein denatured, so it undergoes a conformational change that makes it inactive. Eventually, damage to DNA and cell membranes will disrupt GSH synthesis (Koiram et al., 2007).

Our study also noted a significant enhancement in GSH levels in the radioprotectant agent treatment prior to IR exposure. Significant changes in GSH levels occurred in supplementing temulawak and garlic in liver tissue. While in the spleen tissue, only NAC succeeded in significantly elevated GSH levels. We assume that the active substance curcumin in temulawak is still estimated to be a primary player in the radical scavenging process and trigger an elevated endogenous antioxidant activity. Meanwhile, garlic’s protective effect can be mediated by potent antioxidant compounds such as S-allylcysteine, S-allyl mercaptocysteine, allicin, and selenium (Uzun et al., 2016). In comparison, NAC acts as a source of cysteine and stimulates the formation of GSH, thereby protecting against ROS (Kileiksiz et al., 2008). However, the inability of this radioprotectant agent to increase GSH levels in all tissues may be due to the limited capacity of active antioxidant substances in these tissues.

Fig. 4. Microscopic image of the comet assay results at 1000x magnification in various treatments. Control (A), 6 Gy (B), Temulawak extract + 6 Gy (C), Garlic extract + 6 Gy (D), and NAC + 6 Gy (E).
Supplementation of radioprotective agents had a significant effect on reducing spontaneous DNA damage. The exception occurred in the supplementation of temulawak to Tail DNA parameters, which did not decrease significantly. We think the inability of temulawak to degrade T.DNA is related to the lack of antioxidant capacity in the comet tail region. The percentage level of T.DNA indicates DNA damage (Kumaravel and Jha, 2006). Parallel with our research, Nair and Menon (2013) conducted a study on the consumption of curcumin and ascorbic acid, which can inhibit cellular DNA damage, as evidenced by the lowering in the values of all comet parameters compared to the radiation group. Meanwhile, Srinivasan et al. (2007) revealed that curcumin pretreatment (1, 5, and 10 μg/mL) had a modulatory effect that could inhibit lipid peroxidation and DNA damage in rat hepatocyte cultures.

Furthermore, garlic is prominent in neutralizing free radicals that cause DNA damage. Our previous study confirmed that garlic extract inhibited DNA oxidative damage by forming 8-hydroxy deoxyguanosine (8-OHdG) compounds and γ-H2AX foci (Kisnanto et al., 2020). Belloir et al. (2006) demonstrated that organosulfur compounds have antigenotoxic activity, inhibiting DNA damage in HepG2 cells. Capasso (2013) concluded that garlic has forceful bioactive compounds in allylsulfide derivatives, which have anticancer properties. These compounds can inhibit carcinogenesis and oxidative DNA damage and participate in radical scavenging.

In the present study, NAC also has an important role in the recovery of spontaneous DNA damage by decreasing the values of all comet parameters. Kataoka et al. (2007) demonstrated that NAC reduced IR-induced DNA DSBs formation in human microvascular endothelial cells and oxidative DNA damage in rat liver. DSBs is considered the most significant DNA lesion induced by ionizing radiation.

5 Conclusion

Temulawak extract has a better ability as a radioprotector than garlic extract because it can inhibit lipid peroxidation, stimulate the formation of endogenous antioxidants, and reduce DNA damage. Temulawak extract also has an ability almost identical to NAC in reducing oxidative stress levels in Wistar rats.

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Conflicts of Interest

The authors declare that they have no conflict of interest in relation to this article.

Author contribution statement

T. Kisnonto: Conceptualization, methodology, investigation, and writing the original draft. D. Tetriana: Conceptualization, methodology, and reviewing. D. Yusuf: Conceptualization, methodology, and investigation. Y. Lusiyanti: Methodology and investigation. H.N.E. Surniyantoro: Methodology and investigation. I.K. Hasan Basri: Investigation and reviewing. T. Kisnanto, D. Tetriana, and D. Yusuf are the main contributors to the manuscript.

Ethics approval

The study received ethical approval from the Institutional Animal Care and Use Committee, Indonesia, No. 008/KEPPHP-BATAN/XI/2021, on November 15, 2021.

Informed consent

This article does not contain any studies involving human subjects.

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