

DENTINO
JURNAL KEDOKTERAN GIGI
Vol VIII. No 1. March 2023

**THE EFFECT OF KASTURI LEAF EXTRACT ON THE HEMOGLOBIN LEVEL
EXPOSED TO PERIAPICAL RADIOGRAPHY X-Rays**

Firda Damayanti¹⁾, Didit Aspriyanto²⁾, Juliya Putri Utami³⁾

¹⁾ Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin

²⁾ Department of Dental Radiology, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin

³⁾ Department of Biomedicine, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin

ABSTRACT

Background: Periapical radiographic x-ray exposure can damage living cells, one of which is erythrocytes which contain hemoglobin by forming free radicals. Antioxidant compounds are needed to counteract the effects of these free radicals. One of the natural antioxidant compounds is kasturi leaves which contain flavonoids, triterpenoids and tannins. **Purpose:** To analyze the effect of kasturi leaf extract on hemoglobin levels of male mice exposed to periapical x-ray radiography. **Methods:** This study used a true experimental method with a post test only design with a control group design. The samples used were 24 male mice aged 3-4 months which weighed 20-25 grams. Mice were divided into 4 groups, each group consisted of 6 mice. Group P1 was a group exposed to radiation 1 times a dose of 1 mGy with a dose of 0,2 ml of kasturi leaf extract, group P2 was a group exposed to radiation 7 times a dose of 1 mGy with a dose of 0,2 ml of kasturi leaf extract, Group P3 was a group exposed to radiation 10 times at a dose of 1 mGy with a dose of 0,2 ml of kasturi leaf extract, and group P4 was a group given the extract without radiation exposure. **Results:** The results showed hemoglobin levels in the P1 group = 7.2833 g/dL, the P2 group = 7.4800 g/dL, the P3 group = 12.7800 g/dL, and P4 = 12.4200 g/dL. **Conclusion:** The results showed that there was an effect of kasturi leaf extract on the hemoglobin levels of male mice exposed to periapical x-ray radiography. The results of the Mann Whitney test showed that the results of the P1 and P2 groups were significant to P3 and P4.

Keywords: Antioxidant, kasturi leaf extract, periapical radiography

Correspondence: Firda Damayanti; Faculty of Dentistry, Lambung Mangkurat University, Jl. Veteran No. 128 B, Banjarmasin, South Kalimantan, Indonesia, email: firdadmy@gmail.com

INTRODUCTION

Radiographic examination has a very important role in the field of dentistry. Radiography has a function to help diagnose a disease, determine the state of the disease and plan treatment for a disease in a patient. However, radiography also has negative side effects for the body, namely biological changes to cell death.^{1,2}

Periapical radiography is a type of radiography that is often used in dentistry. Errors in taking periapical radiographs make the operator have to repeat several times until the quality of the resulting photo is of good quality. Repeated radiographic images make the patient repeatedly exposed to radiation, causing an increase in the radiation dose to the patient. According to the International Atomic Energy Agency (IAEA), the recommended dose for periapical radiography is 7 mGy.^{3,4}

Radiation can cause inhibition of the process of blood formation (hematopoiesis) causing the number of blood cells to decrease, one of which is

erythrocytes or red blood cells. Erythrocytes have a very important function in the human body to bind hemoglobin. Hemoglobin has two main functions in the human body, namely transporting oxygen to tissues and transporting carbon dioxide and protons from peripheral tissues to respiratory organs. The normal limit value for Hb levels according to the World Health Organization (WHO) 2001 is for age 5-11 years <11.5 g/dL, age 12-14 years 12.0 g/dl while over 15 years for women > 12.0 g/dL and men > 13.0 g/dL.^{5,6} According to many case studies reported that patients undergoing radiotherapy treatment experienced a decrease in the number of blood cells including hemoglobin.⁷

Exposure to radiation received by the body can interact with water molecules around cells so that the bonds of water molecules are broken and cause the formation of free radicals which will then damage DNA. DNA damage will cause damage to erythrocytes so that hemoglobin is released.⁸ The body that lacks hemoglobin can cause a disease called anemia. Various kinds of

manifestations in the oral cavity can appear in individuals who suffer from anemia. The negative effects of free radicals can be prevented with antioxidant compounds. One type of plant that can be used as a source of natural antioxidants is Kasturi.^{4,9}

Kasturi is one of the typical plant of South Kalimantan. Kasturi is proven to have bioactive compounds that can be used as natural medicines. Based on research on phytochemical tests, kasturi leaves contain phenolic compounds, flavonoids, triterpenoids and tannins.^{10,11} Phenolic compounds are known to have antioxidant activities such as reducing agents, free radical scavengers and electron donors. The flavonoid compounds contained in kasturi leaves are polyphenol compounds that can ward off free radical attacks.¹⁰ Based on this, it is necessary to conduct research on the effect of kasturi leaf extract on hemoglobin levels of male mice exposed to x-ray periapical radiographs.

MATERIAL AND METHODS

This study uses a true experimental design research method with a posttest only design with a control group design that has received ethical approval by the Health Research Ethics Commission, Faculty of Dentistry, Lambung Mangkurat University with No. 031/KEPKG-FKGULM/EC/II/2021. The research was carried out at the Laboratory of FMIPA, Lambung Mangkurat University for plant determination and the Banjarbaru Veterinary Center for the adaptation process, giving extracts, and taking blood from mice. Meanwhile, periapical radiographic irradiation was carried out at the Gusti Hasan Aman Hospital in Banjarmasin and the making of kasturi leaf extract at the BIOCHEMICAL Laboratory of the Faculty of Dentistry, Lambung Mangkurat University. Extracts were made by maceration using 96% ethanol solution. The samples used in this study were 24 male mice with the criteria of having a body weight of 20-25 grams and 3-4 months old which were then divided into 4 groups, each consisting of 6 mice. Group P1 was exposed to periapical radiographic x-ray radiation once with the extract, group P2 was exposed to periapical x-ray radiation 7 times with the extract, group P3 was exposed to periapical radiography x-ray radiation 10 times with the extract, and group P4 was given Kasturi leaf extract without radiation exposure.

The mice were first adapted for 7 days to get the mice used to the new environment. After that, kasturi leaf extract was given for 7 days with a concentration of 10% at a dose of 0.2 ml/head

using a gastric probe. The mice were then taken to the Gusti Hasan Aman Hospital in Banjarmasin by car for irradiation. Periapical radiography x-ray radiation was carried out by placing the mice in a wire cage measuring 11x6x10 cm then given styrofoam to minimize the movement of the mice. Under the mouse cage, a dosimeter was placed to measure the radiographic dose absorbed by the mice. The irradiation was carried out using a single dose of 1 mGy and the dental radiography brand ASAHI. The irradiation was carried out 1, 7, and 10 times with a time lag of each repetition of 1 minute. The mice were then brought back to the Banjarbaru Veterinary Center for the calculation of hemoglobin levels. The calculation of hemoglobin levels was carried out 24 hours after periapical radiographic irradiation. Blood samples was carried out right in the heart of mice as much as 1 ml using a syringe. The blood sample was then placed in a blood tube containing 0.01 ml of 10% EDTA. The calculation of the hemoglobin level of mice was carried out using an automatic hematology analyzer. The results of the study were then analyzed using the *Kruskal Wallis* non-parametric test.

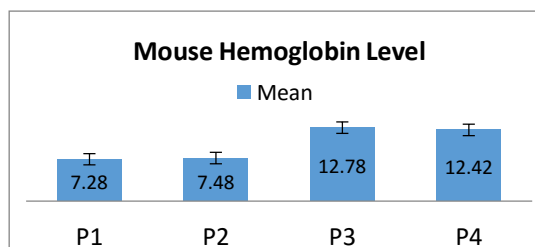
RESULT

This study used 24 male mice which were divided into 4 groups, namely groups P1, P2, P3, and P4. The average radiation dose absorbed by mice in each group was P1=1mGy, P2=7mGy, P3=10mGy. The results of the calculation of hemoglobin levels showed that the lowest hemoglobin level was the P1 group and the highest hemoglobin level was the P3 group.

Table 1. Mean Value and Standard Deviation of Mouse Hemoglobin Level

Group	N	Mean ± SD
P1	6	7.2833 ± 1.16545
P2	5	7.4800 ± 1.19390
P3	5	12.7800 ± 1.38759
P4	5	12.4200 ± 1.43854

Figure 1. Mean Value of Mouse Hemoglobin Level



Groups P1 and P2 showed a decrease in hemoglobin levels in mice, while groups P3 and P4 had an increase in hemoglobin levels and tended to be in the normal value of hemoglobin levels in mice. Then the *Saphiro Wilk* normality test was carried out to find out whether the data was normally distributed or not and the result was p value <0.05, which means the data was not normally distributed. After that, the *Kruskal Wallis* non-parametric analysis test was conducted to determine whether there were significant differences between groups and the *Mann Whitney* test to determine which groups there were significant differences.

Table 2. *Kruskal Wallis* Analysis Test Results

Kruskal Wallis			
	Sum of Squares	df	Sig.
Total	10.584	3	0.014

Table 3. *Mann Whitney* Analysis Test result

Sample Comparison	Sig.
P1-P2	0.776
P1-P4	0.016*
P1-P3	0.012*
P2-P4	0.041*
P2-P3	0.032*
P4-P3	0.919

Based on the results of the *Kruskal Wallis* test, the results obtained p value <0.05 which indicates a significant difference between groups. Meanwhile, based on the results of the *Mann Whitney* test, the group marked with a sign (*) is the group that has a p value <0.05. The p value <0.05 indicates there is a significant difference in the group.

DISCUSSION

In this study, the average value of hemoglobin levels in mice increased from P1 to P4. The lowest hemoglobin level was in the P1 group and increased in the P2 group. However, the average hemoglobin level in both treatments was below the normal hemoglobin value of mice, which was in the range of 7 g/dL. According to Heryanita et al 2018, the normal value of hemoglobin levels in male mice is in the range of 10.7-11.5 g/dL.¹² This is probably because in this group the formation of free radicals due to radiation is not much, but cell damage occurs directly so that the antioxidants of kasturi leaf

extract cannot work. X-ray radiation directly damages erythrocyte cells and damages DNA so that cell damage cannot be minimized by free radicals which cause erythrocyte cell lysis. Periapical X-ray radiography can damage living cells in two ways, directly and indirectly. Periapical radiographic X-rays successfully penetrated the erythrocyte cell membrane of mice, thereby damaging single or double DNA chains and destroying the erythrocyte cell membrane so that the hemoglobin of mice was released. Indirect damage can occur because X-rays react with water molecules in cells to form free radicals.⁶

The process of hematopoiesis begins with the division of stem cells. One stem cell will replace stem cells to produce blood cells, while the other stem cells will differentiate. These early-stage progenitor cells will determine several transcription factors that can shape these cells into certain cell types. Ionizing radiation can reduce the number of blood cells, one of which is erythrocytes in the peripheral circulation. Radiation can reduce the number of immature blood cells (blood cells that are not yet mature) and reduce mature blood cells in the bloodstream. Decrease in blood occurs due to autolysis. The lysed erythrocytes cannot be counted in the erythrocyte count chamber. Meanwhile, necrotic erythrocytes can still be counted because there are still signs of life in the cells.^{13,14}

The decrease in hemoglobin levels in groups P1 and P2 to below the average value of hemoglobin in mice could be caused by several factors. Not only because X-rays directly damage cells, but also because of the possible influence of psychological conditions on mice. In this study, mice were brought from Banjarbaru to Banjarmasin for periapical radiographic irradiation at Gusti Hasan Aman Hospital. The process of traveling far enough allows uncomfortable conditions for mice so that it can affect hemoglobin levels in mice. Decreased hemoglobin levels can also be caused by differences in metabolic rate and immunity levels in mice. so that although given the same treatment, the body's response shown can be different. This also allows the results of data analysis to be abnormal because there are some extreme data caused by the influence of the psychological condition of mice.^{15,16}

Groups P3 and P4 were the treatment groups with a significant increase in hemoglobin levels. The P3 group was the group with the highest average hemoglobin level among the other treatment groups. The P3 group was the treatment group that was exposed to 10 times periapical radiography x-ray radiation and was given kasturi leaf extract. The average value of hemoglobin

levels in the P3 group was 12.78 g/dL. The significant increase in hemoglobin levels in the P3 group could be due to the reaction of the mice's immune system and the higher the dose of radiation exposure, the more free radicals were formed. Thus, the damage that will occur is not due to direct damage but due to the presence of free radicals which can then be reduced by antioxidant compounds. Defense enzymes in the body of mice and flavonoid antioxidants work optimally to detoxify free radicals in the P3 group so that cell damage does not occur.¹⁷ Kasturi leaves contain flavonoid, triterpenoid and tannin compounds. Flavonoids are known to have benefits as natural antioxidants that function to ward off free radicals in the body.¹¹

Free radicals that are formed will cause changes in the structure of DNA and RNA so that mutations or cytotoxicity can occur. Free radicals are unstable so they can disrupt the balance of cells by trying to take electron pairs from other molecules. These highly active free radicals can produce hydrogen peroxide compounds (H₂O₂), superoxide compounds (O₂⁻), and hydroxyl ions (OH) which are also known as Reactive Oxygen Species (ROS). This very large increase in ROS can cause oxidative stress.¹⁸ Flavonoid compounds in kasturi leaf extract can act as antioxidants due to the position and number of large hydroxyl groups. The large number of flavonoid hydroxyl groups is used for scavenging or capturing free radicals that exist. Flavonoids will then donate hydrogen atoms, causing free radicals that were previously unstable to become stable because they have atomic pairs. The process of catching free radicals by antioxidants from kasturi leaves due to radiation can prevent cell damage, one of which is erythrocyte cells so that hemoglobin levels can increase.^{19,20}

The P3 group which was exposed to radiation 10 times and given kasturi leaf extract at a concentration of 10% at a dose of 0.2 ml had an average hemoglobin level which was slightly higher than the P4 group which was only given kastur leaf extract at a concentration of 10% at a dose of 0.2 ml without radiation exposure. This is presumably due to the variation in the number of erythrocytes in each individual. The number of erythrocytes and hemoglobin levels in each individual mouse is influenced by several factors, one of which is physical activity, changes in blood plasma fluid in mice due to dehydration, and nutrition received by mice. The individual differences in the samples used cause the hemoglobin levels in each individual to also differ. This could allow the occurrence of hemoglobin levels in the P3 group slightly higher than the P4

group because the P3 group had a slightly higher hemoglobin level from the start.²¹

This study is in line with research by Rahim et al. 2017 which said that kasturi leaf extract can improve the quality and quantity of white blood cells, namely macrophages in mice. The use of kasturi leaf extract at a concentration of 10% at a dose of 0.2 ml is also a safe dose for mice. This study is also in line with the research of Prisyanto et al, 2014 which stated that the antioxidant effects of Vitamins C and E can prevent damage to wrong blood cells, namely hemoglobin, leukocytes, and platelets even though they are exposed to gamma ray radiation.^{7,22} Based on the results of the study, it can be concluded that there is an effect of kasturi leaf extract on the hemoglobin levels of male mice exposed to periapical radiography x-rays with hemoglobin levels in groups P1 and P2 significant on groups P3 and P4.

REFERENCES

1. Sariarnoferni, Paramita DAV, Mulawarmanti D. Pengaruh Pemberian Alga Coklat terhadap Aktivitas Enzim Katalase Kelenjar Submandibularis Rattus Rendah. *Quanun Medika*. 2017; 1(2): 2-4.
2. Septina F dan Reyfaldo R. Perbedaan Kualitas Radiografi Periapikal antara Film Konvensional dan Film Instan di Instalasi Radiologi FKG Universitas Brawijaya Malang. *Jurnal Radiografi Dentomaksilofasial*. 2020; 4(1): 45.
3. Masserat V, Ebrahimi HS, Eil N, Mollashasi J, Naebi M. 2017. Evaluation of Frequency of Periapical Radiographic Errors in Dental Radiology Departement in Zahedan in 2014-*Scholars Journal of Applied Medical Sciences (SJAMS)*. 2015; 5(1B): 112-115
4. Mersil S, Pradono SA. Manifestasi Klinis Rongga Mulut sebagai Penanda Awal Penyakit Iron deficiency Anemia (IDA). *Jurnal Ilmiah dan Teknologi*. 2017; 13(2): 1-2.
5. Noorma, Sukmana BI, Saputera D. The Influence Between the Length of Radiographer Working Time and Reduction of Salivary pH. *Dentino (Jurnal Kedokteran Gigi)*. 2018; 3(1): 127.
6. Gunadi VIR, Mewo YM, Tiho M. Gambaran Kadar Hemoglobin pada Pekerja Bangunan. *Jurnal e-Biomedic (eBm)*. 2016; 4(2): 2.
7. Prisyanto R, Santoso DR, Juswono UP, Cahyati Y. Pengaruh Pemberian Kombinasi Vitamin C dan E terhadap Jumlah Hemoglobin,

- Leukosit dan Trombosit Pasca Iadiasi Sinar Gama. *Natural B*. 2014; 2(3): 290-295.
8. Darlina dkk. Efek Jenis Pekerjaan, Usia dan Gender Terhadap Kerusakan DNA Sel Limfosit Pekerja Radiasi Medis. *Jurnal Biotek Medisiana Indonesia*. 2018; 7(1): 39.
 9. Muthia R, Saputri R, Verawati SA. Uji Aktivitas Antioksidan Ekstrak Etanol Kulit Buah Mundar (*Garcinia forbesii* King.) Menggunakan Metode DPPH (2,2 Diphenyl-1-Picrylhydrazil). *Journal Pharmascience*. 2019; 6(1): 75.
 10. Marliani L, Naimah A, Roni A. Penetapan Kadar Fenolat Total dan flavonoid Total Ekstrak Etanol Daun, Kulit Batang dan Kulit Buah Kasturi (*Mangifera casturi*). *Prosiding Seminar Nasional Tumbuhan Obat Indonesia ke-50*. Samarinda; 2016. p. 275-281.
 11. Tanaya V, Retnowati R, Suratmo. Fraksi Semi Polar dari Daun Mangga Kasturi (*Mangifera casturi* Kosterm). *Kimia Student Journal*. 2015; 1(1): 783.
 12. Heryanita Y dkk. The Value of erythrocytes, Hemoglobin, and Hematocrit of Mice (*Mus musculus*) Exposed to Cigarette Smoke and Given Red Watermelon Extract (*Citrullus vulgaris*). *Jurnal Medika Veterina*. 2018; 12(1): 29.
 13. Erma NK dan Supriyadi. Penurunan Jumlah Eritrosit Darah Tepi Akibat Paparan Radiasi Sinar-x Dosis Radiografi Periapikal. *Stomatognatic JKG Unej*. 2012; 9(3): 140-144.
 14. Febriani A dan Rahmawati Y. Efek Samping Hematologi Akibat Kemoterapi dan Tatalaksananya. *Jurnal Respirasi*. 2019; 5(1): 24.
 15. Katari HL, Getas IW, Resnheleksmana E. Lama Paparan Asap Rokok terhadap Kadar Hemoglobin (Hb) pada Hewan Coba Tikus Putih (*Rattus norvegicus*) Galur Wistar. *Jurnal Analisis Medika Bio sains*. 2017; 4(2): 79.
 16. Setyaningsih E. Kadar Hemoglobin Tikus Putih (*Rattus norvegicus* L) Jantan Strain Wistar yang Terdedah Asap Rokok Filter dan Non Filter. *Seminar Nasional Pendidikan Biologi dan Saintek II* . 2017; 573(82): 736.
 17. Fakhurreza M dan Majidah PK. Pengaruh banyaknya radiasi dan Perubahan Energi Sinar-X terhadap Peningkatan Pembentukan Radikal Bebas pada Air. *Journal of Health Studies*. 2018; 2(1): 37.
 18. Ardiny K, Supriyadi, Subiyantoro S. Jumlah Sel pada Isolat Monosit Setelah Paparan Tunggal Radiasi Sinar-X dari Radiografi Periapikal. *e-Jurnal Pustaka Kesehatan*. 2014; 2(3): 567.
 19. Adelia KAC, dkk. Pengaruh Pemberian Radiasi Gamma dan Ekstrak Kulit Manggis (*Garcinia mangostana* L) terhadap Kadar SGPT Organ Hati Mencit (*Mus musculus*). *Journal For Physics Education and Applied Physycs*. 2020; 2(2): 77-78.
 20. Sukendro SJ, Sulistijarso N, Aryati E, Hendari R. Efektivitas Larutan Kulit Buah Naga Merah (*Hylecereus polyrhizus*) terhadap Jumlah Koloni Bakteri di Saliva. *Jurnal Kesehatan Gigi*. 2015. 2(1): 58-63.
 21. Nuradi dan Jangga. Hubungan Kadar Hemoglobin dan Nilai Hematokrit pada Perokok Aktif. *Jurnal Media Analisis Kesehatan*. 2020; 11(2): 151-152.
 22. Rahim MA, Suartha IN, Sudimartini LM. Efek Imunostimulator Ekstrak Daun Kasturi (*Mangifera Casturi*) pada Mencit. *Indonesia Medicus Veterinus*. 2017; 6(1): 12-13.