HAYATI
Journal of Biosciences

Vol. 31 No. 6, November 2024 1061-1070
DOI:10.4308/hjb.31.6.1061-1070
ISSN: 1978-3019
EISSN: 2086-4094

Extract of Red Okra Pod (*Abelmoschus esculentus* L. Moench) Chemoprevents *N*-Methyl-*N*-Nitrosourea-Induced Kidney Proximal Tubular Cells Damage

Divany Hunaimatul Achhlam[1], Sri Puji Astuti Wahyuningsih[1*], Lukiteswari Dyah Tri Hapsari[2], Hari Soepriandono[1], Firli Rahmah Primula Dewi[1]

[1]*Department of Biology, Faculty of Sciences and Technology, Universitas Airlangga, Surabaya 60115, Indonesia*
[2]*Faculty of Medicine, Universitas Airlangga, Surabaya 60132, Indonesia*

ARTICLE INFO

Article history:
Received July 25, 2023
Received in revised form December 20, 2023
Accepted May 22, 2024

KEYWORDS:
Antioxidant enzyme,
N-Methyl-*N*-Nitrosourea,
oxidant,
red okra,
tubular proximal cells

ABSTRACT

N*-Methyl-*N*-Nitrosourea (MNU) is a compound that induces oxidative stress resulting in high levels of oxidants and damage to body cells. Red okra contains polyphenol and flavonoid active ingredients as antioxidants. This study aims to analyze the effect of ethanol extract of red okra pods (ROPE) on oxidant levels, antioxidant enzymes, and damage to rat kidney proximal tubule cells due to NMU. This study used 30 rats divided into six treatment groups, namely CN (normal), N (negative control; MNU 50 mg/kg BW), P (positive control; MNU and MTX 50 mg/kg BW), T1 (MNU and ROPE 50 mg/kg BW), T2 (MNU and ROPE 100 mg/kg BW), and T3 (MNU and ROPE 200 mg/kg BW). The treatment was carried out on all groups after eight weeks. The results indicate that malondialdehyde (MDA) and nitrogen oxide (NO) decrease with the ROPE treatment. The glutathione reductase (GSH) activity as an antioxidant enzyme increased T1 and T2, while glutathione peroxidase (GPx) showed an increase in T2 and T3. Furthermore, the biochemical marker of the rat kidney showed lower blood urea nitrogen (BUN) and creatinine (Cre) levels in all treatment groups. Then, the repair of damaged proximal tubule cells showed an increase in normal cells and lower swollen cells; however, there was a degradation in necrotic cells in T2 and T3. It can be indicated that the ROPE can act as an antioxidant that can reduce MDA and NO levels, increase GSH and GPx levels, and reduce damage to proximal renal tubule cells due to MNU.

1. Introduction

N-Methyl-*N*-Nitrosourea (MNU) is a carcinogenic compound that can relate its carcinogenic properties to oxidative stress regulators. *N*-Methyl-*N*-Nitrosourea is a compound of the *N*-nitroso group. Nitroso compounds are alkylating agents that can react with biomolecules, such as DNA and RNA nucleic acids. The agents that react with DNA, causing DNA damage, will create wrong nucleotide incorporation during DNA replication and consequently in RNA transcription, which has an important role in mutagenesis and carcinogenesis processes (Faustino-Rocha *et al.* 2015). Nitroso compounds can be found in cured meats, beer, sauces, and cheese. In addition to food, nitroso can also be found in cigarette smoke and cosmetics. The accumulation of these carcinogenic agents can cause oxidative stress, which is the main factor of premature aging and various other chronic diseases, such as organ fibrosis, organ cancer, kidney failure, and kidney necrotic cells (Valko *et al.* 2006).

Oxidative stress can be defined as an increased level of reactive oxygen species (ROS). ROS can affect various diseases, such as hypertension, atherosclerosis, diabetes mellitus, coronary heart disease, stroke, and other chronic diseases (Paravicini and Touyz 2008). The uncontrolled concentration of ROS by internal defense mechanisms such as antioxidants or enzymes involved in oxygen radical scavenging, among others, superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSH), and glutathione peroxidase (GPx). Besides that,

* Corresponding Author
E-mail Address: sri-p-a-w@fst.unair.ac.id

oxidative damage occurs to proteins, lipids, and DNA, which could lead to cytotoxicity, genotoxicity, and even carcinogenesis when damaged (mutated) cells can proliferate (Gagné 2014). Oxidative stress emerges when an imbalance exists between free radical formation and the capability of cells to clear them. For instance, excess hydroxyl radical and peroxynitrite can improve lipid peroxidation, thus damaging cell membranes and lipoproteins. This will lead to malondialdehyde (MDA), one of the markers when cell damage occurs (Pizzino 2017).

The magnification in ROS affects the number of free radicals in the body so that it can evoke various tissue damage, including kidney tissue. According to Chevalier (2016), tubules are the part of the kidney susceptible to cellular damage. It can occur due to the accumulation of toxic materials in the tubules and the weak and leaky character of the tubular epithelium. In addition, the proximal convoluted tubule contributes to fluid, electrolyte, and nutrient homeostasis by reabsorbing approximately 60–70% of the water and NaCl (Curthoys and Moe 2014).

Administration of certain amounts of MNU can also lead to necrotic in the kidneys. Necrotic commonly occurs in the proximal convoluted tubule or is called acute tubular necrotic, which can provoke kidney failure. One of the prognostic biomarkers of kidney failure is the number of residual substances in the blood that should be excreted through urine has elevated, such as blood urea nitrogen (BUN) and creatinine (Cre). The BUN in chronic kidney disease (CKD) is the accumulation of uremic toxin nitrogen that can be involved in CKD anemia (Kim *et al.* 2021). In addition, research by Tsuruma *et al.* (2012) and Adefisan *et al.* (2018) concluded that MNU can bring about cellular damage by producing ROS in amounts of 100, 500, and 1,000 g/ml. The exposure can multiply lipid peroxidation by lowering the enzymatic defenses, namely GPx, glutathione-s-transferase (GST), and CAT, as well as decreasing GSH. The kidneys excreted Cre by glomerular filtration and proximal tubular secretion. If kidneys are exposed to toxic substances, it will damage the kidney structure and reduce the glomerular filtration rate and the proximal convoluted tubule absorption ability, which can heighten Cre levels in the blood, where enhanced levels of creatinine in the blood can be an indication of reduced kidney function (Fevrier-Paul *et al.* 2018). Normal blood Cre levels in mice are 14.2, 5.4, and 9.2 mol/L with Jaffé, enzymatic, and HPLC methods (Palm and Lundblad 2005).

Red okra (*Abelmoschus esculentus* L. Moench) is a plant from the Malvaceae family commonly used in various traditional medicines. Husen *et al.* (2020) state that okra extract has antioxidant levels to reduce oxidative stress in renal proximal tubule cells with prolonged hyperglycemic conditions. Red okra contains antioxidants, neuroprotective, antidiabetic, antihyperlipidemic, and anti-inflammatory compounds and also consists of polyphenol and flavonoid compounds as active constituents (Ullah *et al.* 2020). This is supported by the results of Wahyuningsih *et al.* (2020) research related to the antioxidant and nephroprotective effect of okra pod extract against lead acetate-induced toxicity in mice, okra pod has an IC_{50} value of 35.21 g/ml so it can be categorized as a very strong antioxidant. Red okra (100 g) contains several nutrients, namely 2.44 g crude protein, 2.11 g deoxidized sugar, 0.682 g carotene, 1.06 g cellulose, 10.2 mg vitamin B, 1.25 mg vitamin A, 26.5 mg of vitamin C, and several other minerals which are higher than other vegetables or fruit. Which vitamin C as an antioxidant will neutralize free radicals by donating electrons to free radicals (Liu *et al.* 2008). Red okra fruit also contains seeds and mucus, which contain flavonoids as antioxidant compounds in the form of quercetin and isoquercetin, which are derivatives of quercitrin (Chaemsawang *et al.* 2019). Isoquercitrin exhibits many biological activities, including antioxidant activity, inhibiting inflammation, and suppressing ROS production to prevent cell damage due to oxidative stress (Qiu *et al.* 2019), especially in renal proximal tubule cells.

This study aimed to analyze the effect of red okra (*Abelmoschus esculentus* L. Moench) pod ethanol extract (ROPE) on levels of oxidants, antioxidants enzyme, and cellular damage to rat kidney proximal tubule cells induced by MNU. In this study, we use the indicators of MDA, NO, GSH, GPx, BUN, and Cre levels as biomarkers and the parameters of the kidney's cellular damage. Based on Wahyuningsih *et al.* (2020), the optimal concentration of okra methanol extract to reduce oxidant levels, increase antioxidant enzyme levels, and reduce the number of swollen and inflammatory cells in hepatocytes is 50–100 mg/kg BW. Therefore, in this study, ROPE was administered using three concentrations, namely 50, 100, and 200 mg/kg BW, to see whether a higher ROPE concentration could still function optimally or not. The use of ethanol as a

solvent is because ethanol is a universal solvent that is polar in nature, so it is hoped that it can attract polar and semipolar compounds in the crude extract of red okra fruit like polyphenol and flavonoid compounds. Both groups of compounds can act as antioxidants.

2. Materials and Methods

2.1. Ethical Clearance

This study has been approved by the Health Research Ethical Clearance Commission of the Faculty of Dental Medicine, Universitas Airlangga, with registration number 270/HRECC.FODM/V/2022.

2.2. Chemicals and Reagents

Ethanol, MNU, Elabscience® GSH or GPx Activity Assay Kit (Hayward, California, USA), Bioxytech® NO-586 spectrophotometric assay kit was purchased from Oxis International, Inc. (Portland, Oregon, USA), neutral-buffer, 10% formalin, entellan, paraffin, alcohol hematoxylin, and eosin were purchased from a Faculty of Pharmacy Airlangga University, Surabaya, Indonesia. The red okra pod was purchased from Rendy Farm Malang, East Java, Indonesia, identified and verified by the Indonesian Institute of Sciences (BRIN). The study used chemical reagents that were of pure analytical grade.

2.3. Extraction of Plant Material

The ROPE was made using ethanol solvent extraction. Red okra pod cleaned and thinly sliced. Then, the red okra pieces were dried in a shady place under the sun for 14 days. Fragments of red okra pod were mashed with a grinding machine to filter the resulting red okra pod powder through a number 40 sieve. The filtered results were stored in an airtight container at a temperature of -20°C. The powdered red okra pod (500 g) was dissolved in 96% ethanol solvent and then sonicated for one h. The solution was extracted with a Soxhlet extractor (1,000 ml) for 72 h at room temperature while stirring continuously. The red okra pod extract was filtered and evaporated at a temperature of 30°C in a rotatory evaporator to obtain a solid crude extract. Next, the obtained ROPE crude extract was freeze-dried to remove solvent and stored in a desiccator.

2.4. Animal and Experimental Design

Thirty female rats (*Rattus norvegicus*) aged 4-8 weeks, weighing ±180 g, were acclimatized for two weeks. Rats are placed in plastic tubs (one rat per tub) closed with wire at room temperature and a lighting system of 12 hours of light and 12 hours of darkness in the Faculty of Science and Technology animal laboratory at Universitas Airlangga, Surabaya, Indonesia. The rats had free access to food and water during treatment. Rats were divided into six groups, each consisting of five mice. The administration of MNU was carried out in the first four weeks after acclimatization. The normal control group (CN) was given 0.5 ml of saline. The negative control group (N), the positive control group (P), and the treatment groups (T1, T2, and T3) were given MNU with a single dose of 50 mg/kg BW (Gal *et al.* 2020). The administration of ROPE to rats was carried out after MNU to the treatment group with amounts of 50 mg/kg BW (T1), 100 mg/kg BW (T2), and 200 mg/kg BW (T3) (Wahyuningsih *et al.* 2020).

2.5. Serum and Kidney Collection

After eight weeks of treatment, the rats' blood serum and kidneys were collected. Rat anesthetized using 10% ketamine in a glass jar. Rat blood (5 ml) was taken from the heart using a disposable syringe. The blood was collected in a non-airtight falcon tube and incubated at room temperature on a slope for 2 h. The blood was centrifuged at 3,000 rpm for 10 min to obtain serum. The rat was dissected, and the right kidney was removed using surgical instruments. That kidney was cleaned with a standard saline solution. The kidney was cut into two parts and immersed in a 10% formalin buffer solution in a sample bottle.

2.6. Determination of NO and MDA

The NO was determined by sulphanilic acid, phosphoric acid, and N-(1-naphthyl)-ethylenediamine, whilst the MDA level was determined using Bioxytech® MDA-586 spectrophotometric assay kit (Wahyuningsih *et al.* 2020). MDA was measured at wavelengths 586 nm, and NO was calculated at absorbances 540 nm using a microplate UV-vis spectrophotometer S-22, BOECO, Hamburg, Germany.

2.7. Determination of GSH and GPx

The GSH and GPx as antioxidant enzyme activity were measured with Elabscience® GSH or GPx Activity Assay Kit according to the kit procedure. The OD values were read using a UV-vis spectrophotometer at 412 nm.

2.8. Determination of BUN and Cre

The concentration of BUN was measured with Diagnostic System Kit for Response®: Urea FS. The optical density was read using a UV-vis spectrophotometer at 343 nm. Creatinine concentration was measured with the Diagnostic System Kit for Respon®: Creatinine FS. The optical density was read using a UV-vis spectrophotometer at 412 nm.

2.9. Histological Examination of The Kidney

Preparation of kidney histology preparations uses the Kiernan method. One of the kidney pieces was washed with running water for one night. Then, the kidneys are processed with alcohol and xylol. Next, the kidneys were embedded in paraffin. The kidneys were excised in paraffin using a microtome with a thickness of 4 mm. The pieces are attached to an albumin-covered glass slide. Kidney histology preparations were stained using Haematoxylin-Eosin (HE) staining and covered with entellan and a cover slip. Kidney observation was made on an Olympus CX23 light microscope in Shinjuku, Tokyo, Japan, at the magnification of 400x. All photos were taken with an Olympus DP-10 digital camera in Shinjuku, Tokyo, Japan. Tubular damage was evaluated according to the method reported by Okada *et al.* (2015) from twenty microscopic fields obtained from each group. The tubular proximal cell was grouped as necrotic, normal, and swollen tubular epithelial cells. The number of cells was counted using graticulae and was expressed as the percentage (%) of the total cells counted.

2.10. Data Analysis

The data from this study were analyzed with statistical tests for the IBM Statistical Package for the Social Sciences (SPSS) 25.00, Armonk, New York, USA, for the Windows program. Nitric oxide and MDA level data were tested using the Brown-Forsythe test and LSD ($p<0.05$). Meanwhile, data on GSH, GPx, BUN, Cre levels, and damage to proximal renal tubule cells were tested using One Way ANOVA and Duncan's test ($p<0.05$).

3. Results

3.1. The Effect of ROPE on NO and MDA

The effect of ROPE on the concentration of the NO and MDA can be seen in Figure 1. The administration of MNU to the negative control group that was only given MNU (N) showed a significant increase in the concentration of the NO compared to the CN and all the treatment groups (T1, T2, and T3). Meanwhile, the use of ROPE showed a significant decrease in the concentration of NO in treatment group 1

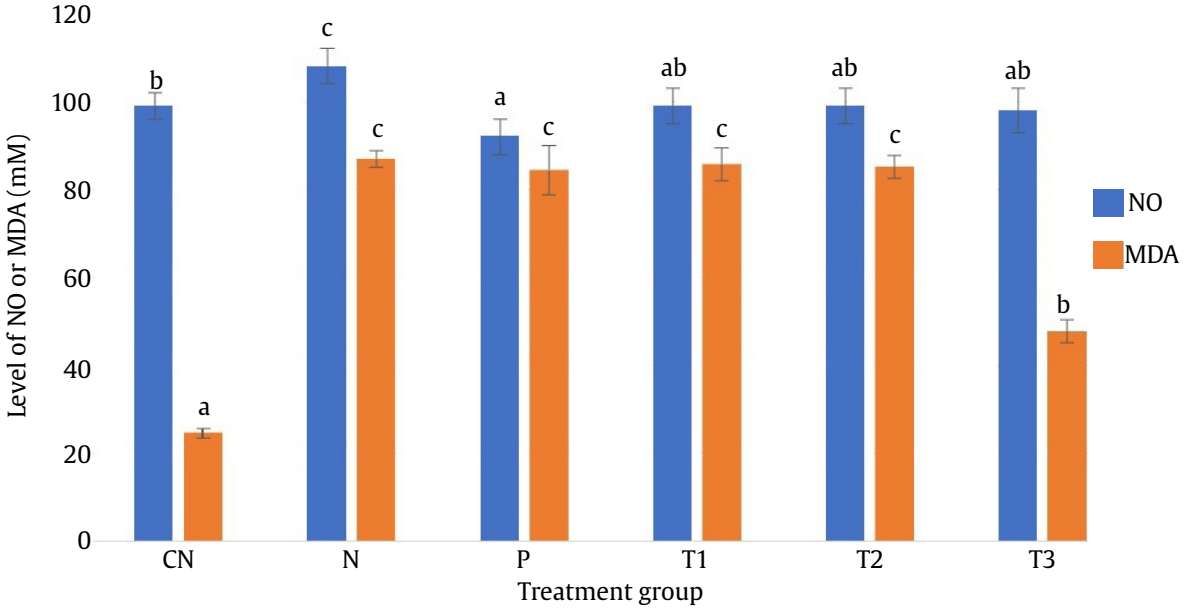

Figure 1. The effect of red okra ethanol extract to decrease levels of oxidant NO and MDA. CN; Rat was not given MNU and ethanol extract of red okra, N; Rat was given 50 mg/kg BW MNU alone, P; Rat was given 50 mg/kg BW MNU and MTX, T1, T2, and T3; Rat was given 50 mg/kg BW MNU and 50, 100, and 200 mg/kg BW ethanol extract of red okra, respectively. a, b, c Different superscript within each figure indicates a significant difference between the means ($p<0.05$)

(T1) with a dose of 50 mg/kg BW compared to the normal control group (CN). The administration of MNU to the negative control group (N) showed a non-significant increase in the MDA concentration compared to the normal control group (CN). The administration of ROPE showed a significant decrease in the concentration of the MDA in treatment group 3 (T3) with a dose of 200 mg/kg BW compared to the negative control group (N), P, T1, and T2.

3.2. The Effect of ROPE on GSH and GPx Activity

The effect of ROPE on the activity of the antioxidant enzymes GSH and GPx can be seen in Table 1. The administration of MNU to the negative control group showed a not significant decrease in the antioxidant enzyme GSH activity compared to the normal control group and treatment group 3 (T3); however, it showed a significant increase in treatment group 1 and 2 (T1 and T2). The administration of MNU to the negative control group showed a significant decrease in the antioxidant enzyme GPx activity compared to the P, T2, and T3; however, it showed a non-significant with the T1 and CN. The administration of ROPE showed a non-significant put on in the activity of the antioxidant enzyme GPx in T2 and T3.

3.3. The Effect of ROPE on BUN and Cre

The effect of ROPE on the concentration of the BUN and Cre can be seen in the Table 2. The presence of MNU in the negative control group that was only given MNU (N) showed a significant increase in the concentration of the BUN compared to the normal control group (CN). The administration of ROPE showed a significant bring down in the concentration of the BUN in all treatment groups (T1-3) and was still higher than the normal control (CN). The administration of MNU to the negative control group (N) showed a significant increase in the concentration of Cre compared to the normal control group (CN). The administration of ROPE showed a significant decrease in the concentration of Cre in all treatment groups.

3.4. Histopathological Examination

The effect of ROPE on normal, swollen and necrotic cell can be seen in Figure 2. Giving MNU to N showed an insignificant decrease in the percentage of normal cells compared to CN. Still, it showed a significant increase in the percentage of swollen cells and necrotic cells compared to CN. The presence of MTX as a chemotherapy drug on P showed a significant increase in the percentage of normal cells compared to N but an insignificant increase in the percentage of normal cells compared to CN. The administration of MTX to P showed a significant lower in the percentage of swollen cells and the percentage of necrotic cells compared to N. Still, it showed a significant promotion in the percentage of swollen cells and the percentage of necrotic cells when compared to CN. The administration of ROPE at T1 showed a significant reduction in

Table 1. The effect of red okra pod ethanol extract on increased GSH and GPx levels

Treatments	GSH level (mg/L)	GPx level (U/ml)
CN	98.58^{a}±24.65	183.19^{ab}±21.29
N	111.54^{a}±25.36	149.36^{a}±13.03
P	243.89^{d}±24.52	230.43^{c}±17.19
T1	156.57^{b}±9.86	178.72^{ab}±22.25
T2	198.85^{c}±13.36	205.85^{b}±21.86
T3	110.43^{a}±18.64	207.77^{bc}±20.13

$^{a, b, c}$Different superscript within each figure indicates a significant difference between the means ($p<0.05$). GSH: glutathione reductase, GPx: glutathione peroxidase, CN; Rat was not given MNU and ethanol extract of red okra, N; Rat was given 50 mg/kg BW MNU alone, P; Rat was given 50 mg/kg BW MNU and MTX, T1, T2, and T3; Rat was given 50 mg/kg BW MNU and 50, 100, and 200 mg/kg BW ethanol extract of red okra, respectively

Table 2. The effect of red okra pod ethanol extract to reduce BUN and Cre levels

Treatments	BUN (mg/dl)	Cre (mg/dl)
CN	32.06^{a}±4.35	0.71^{b}±0.14
N	54.41^{d}±6.50	1.02^{c}±0.13
P	44.41^{bc}±5.32	0.63^{ab}±0.22
T1	44.82^{bc}±6.08	0.64^{ab}±0.24
T2	49.37^{cd}±5.48	0.47^{ab}±0.17
T3	40.03^{b}±4.09	0.43^{a}±0.14

$^{a, b, c}$Different superscript within each figure indicates a significant difference between the means ($P<0.05$). BUN; Blood urea nitrogen, Cre; Creatinine, CN; Rat was not given MNU and ethanol extract of red okra, N; Rat was given 50 mg/kg BW MNU alone, P; Rat was given 50 mg/kg BW MNU and MTX, T1, T2, and T3; Rat was given 50 mg/kg BW MNU and 50, 100, and 200 mg/kg BW ethanol extract of red okra, respectively

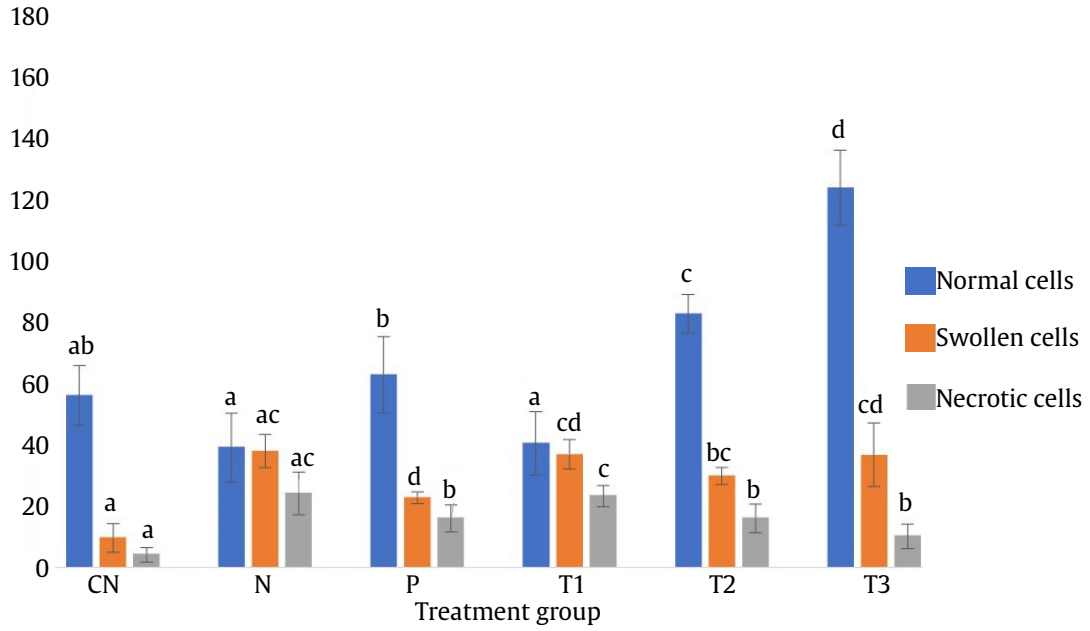

Figure 2. The effect of ethanol extract of red okra pod in normal cell density (blue), swollen cell density (orange), and necrotic cell density (grey) in one field. CN; Rat was not given MNU and ethanol extract of red okra, N; Rat was given 50 mg/kg BW MNU alone, P; Rat was given 50 mg/kg BW MNU and MTX, T1, T2, and T3; Rat was given 50 mg/kg BW MNU and 50, 100, and 200 mg/kg BW ethanol extract of red okra, respectively. a, b, c, d Different superscripts within each figure indicate a significant difference between the means ($p<0.05$)

the percentage of normal cells compared to P. It showed no significant decrease when compared to CN and N. The percentage of swollen cells and the percentage of necrotic cells in T1 showed an insignificant drop when compared to N but showed a significant when compared to CN. Compared with P, the percentage of swollen cells in T1 showed an insignificant accrue but a significant append in the percentage of necrotic cells. The administration of ROPE at T2 and T3 showed a significant increase in the percentage of normal cells compared to CN, N, and P. The percentage of swollen cells in T2 showed an insignificant degradation compared to N but showed a significant enhancement compared to CN and P. The percentage of swollen cells in T3 showed an insignificant drop when compared to N but showed a significant elevate when compared to CN and showed a significant height when compared to P. At T1, T2, and T3, there was a significant difference in the percentage of normal and necrotic cells, but there was an insignificant difference in the percentage of swollen cells. Giving MTX as a chemotherapy drug to P can enlarge the percentage of normal cells and reduce the percentage of swollen cells and the percentage of necrotic cells, as in the giving of ROPE with a specific dose.

The following is a histological picture (Figure 3) of the proximal convoluted kidney tubule in each treatment group that showed signs of tissue damage, such as swollen and necrotic cells.

4. Discussion

The administration of MNU as a carcinogenic agent can cause tumors. According to Gal *et al.* (2020), the administration of MNU at a dose of 50 mg/kg BW causes tumors in the mammary gland area and other organs around it. The nitroso compound in MNU is one of the carcinogenic compounds from the *N*-nitrosamine group. The precursors for nitroso formation are NO and secondary amines (Drabik-Markiewicz *et al.* 2009). MNU can also involve oxidative stress by increasing ROS. Low NO levels (<1 M) play a role in signal transduction pathways and mediate physiological processes. High NO levels (>1 M) can react with other ROS to produce significantly high RNS levels that generate cell damage through lipid peroxidation mediated by free radicals, thiols, amine nitration, tyrosine nitration, and DNA deamination (Scott *et al.* 2021). Normal levels of NO range from 0.15 to 2.2 mol/kg (Luiking *et al.* 2010) , which have several benefits for

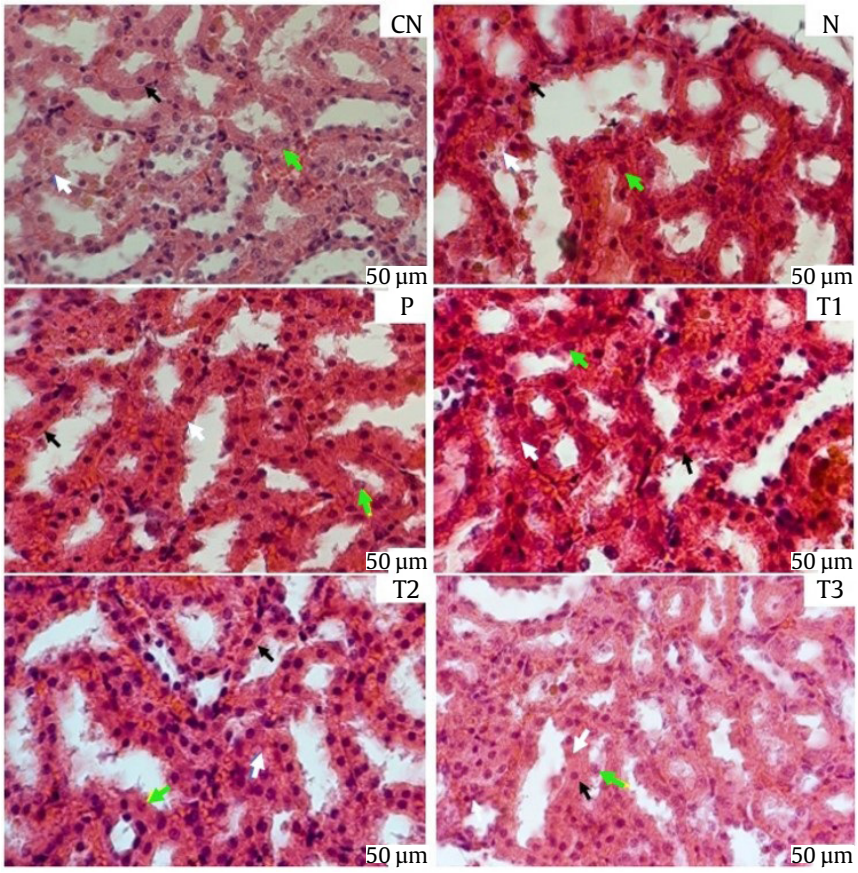

Figure 3. Histopathological view of rat kidney sections. CN; Rat was not given MNU and ethanol extract of red okra, N; Rat was given 50 mg/kg BW MNU alone, P; Rat was given 50 mg/kg BW MNU and 50 mg/kg BW MTX, T1, T2, and T3; Rat was given 50 mg/kg BW MNU and 50, 100, and 200 mg/kg BW ethanol extract of red okra, respectively using hematoxylin and eosin stain technique (×400). Black arrow: a normal cell, white arrow: necrotic cell, and green arrow: swollen cell deviation

the body, such as killing foreign agents and relaxing the smooth vascular muscles, respiratory tract, gastrointestinal tract, and uterus. MNU did change the production of NO and bring out an extent of around 15–20% in lipopolysaccharide-induced NO production regulation (Moon 2010). Methotrexate is an anti-metabolite of folic acid that can act as an anticancer (chemotherapeutic drug) commonly used for therapy in various cancers that does not directly inhibit cells or block related enzymes, but MTX will inhibit the conversion of dihydrofolate to tetrahydrofolate (THF), which functions as a coenzyme in several transmethylation reactions in the synthesis of purine and thymidine nucleic acids, as well as DNA replication or repair (Tian and Cronstein 2007). The inhibition of intracellular THF production by MTX will lead to disturbances in cell proliferation and metabolism (Koźmiński *et al.* 2020). Therefore, MTX can suppress the process of elevated ROS production in cancer cells by stopping the growth of cancer cells (Carew *et al.* 2003).

The administration of MNU can increase ROS (increase lipid peroxidation), which can reduce the enzymatic defenses such as inhibiting the antioxidant enzymes (GPx) action (Adefisan *et al.* 2018). High NO levels in cells can inactivate GPx as an antioxidant enzyme by binding directly to amino acid residues in the GPx molecule, according to Rasool *et al.* (2015) which states that the increased levels of oxidative stress (MDA) and demote enzymatic antioxidants (SOD, GPx, CAT, and GSH) reflect the pathological conditions. Damage to the kidneys will induce BUN to accumulate in the blood as a by-product of protein metabolism when the glomerular filtration rate (GFR) drops (Xue *et al.* 2014). Blood urea nitrogen functions as a biomarker released directly into the blood or urine by the kidneys in response to injury, which may be an early marker of drug-induced renal toxicity (Griffin *et al.* 2019). There was an extension in blood Cre levels of mice with breast cancer exposed to MNU. The promoted Cre levels in mouse

blood serum due to the antineoplastic activity of MNU are associated with an apoptotic mechanism that begins when there is excessive accumulation of DNA damage in cells that are highly sensitive to its action. MNU, a carcinogenic compound, can damage the kidney structure, unload the glomerular filtration rate, and absorb the proximal convoluted tubule, which can enhance Cre levels in the blood (Abou Zaid *et al.* 2017). The administration of MNU can decrease the percentage of normal tubular cell density and increase the percentage of swollen and necrotic cell density. Cell swelling is a way for cells to adapt to repair damage or environmental changes Wahyuningsih *et al.* (2020) which can also occur due to extended oxidative stress and lipid peroxidation that can reduce cell membrane permeability. The degraded cell membrane permeability disrupts the sodium-potassium ion transport mechanism, which causes irreversible cell swelling and non-irreversible necrotics. This is confirmed by Ansori *et al.* (2019) that free radicals give rise to lipid peroxidation that can damage the structure of cell membranes and the structure and function of the renal proximal tubule cells. Increased cell necrosis can be caused by MNU toxicity, which can cause DNA damage and cell damage Tsubura and Yoshizawa (2014). Necrotic is cell death with no chromatin in the nucleus cell, wrinkled (no longer vesicular), the nucleus looks denser, the dark black (pyknosis), the nucleus becomes fragmented (karyorrhexis), invisible, and pale colors (karyolysis). The increased density of swollen cells and necrosis is also accompanied by increased BUN and Cre levels due to decreased kidney function and increased oxidative stress. The enhancement in plasma Cre levels could climb to approximately twice the normal level if there is a degradation in kidney function by up to 50% (Nijveldt *et al.* 2001).

Abelmoschus esculentus contains antioxidant compounds (flavonoid and polyphenol) such as quercetin and isoquercetin (Chaemsawang *et al.* 2019) with DPPH test results (Majd *et al.* 2019); Wahyuningsih *et al.* 2020) as the highest antioxidant activity with an IC_{50} value of 35.21 g/ml (powerful antioxidant). This study uses red okra as it contains higher flavonoids than green okra (Zainuddin *et al.* 2022). Syam *et al.* (2020) state that the flavonoid content of red okra fruit has a value of (14.243±0.084) mg quercetin/5 g simplicial, which is higher than the flavonoid content of green okra fruit, which is worth (12.878±0.078) mg quercetin/5 g simplicial. In addition, the polyphenol content of red okra fruit had a higher value of (45.081±0.0837) mg tannic acid/5 g simplicial compared to the polyphenol content of green okra fruit, which was worth (33.553±0.785) mg tannic acid/5 g simplicial. Flavonoids and polyphenols in red okra can stabilize ROS by capturing free radicals directly by donating their hydrogen atoms which can raise the antioxidant enzyme activity (Akhlaghi and Bandy 2009). This study used ethanol as a solvent in the crude extract of red okra because it is a polar universal solvent that can attract polar and semipolar compounds present as an antioxidant. Antioxidant compounds in ROPE can reduce NO levels, which can stop and eliminate oxidative stress by completing the oxidation-reduction reaction of active metal ions and breaking the chain of free radical reactions (Ahn-Jarvis *et al.* 2019). Plants that have a role as antioxidants have strong inhibitory activity on NO production in cells. According to Wahyuningsih *et al.* (2020), the methanol extract of red okra pod can reduce NO levels to normal conditions. Red okra extract can reduce the biochemical parameters of liver damage, the number of necrotic liver cells, and swelling hepatocytes (Wahyuningsih *et al.* 2021). Phytochemical studies show that red okra consists of polyphenolic compounds and active flavonoids (Xia *et al.* 2015). Flavonoids as anti-inflammatory have a mechanism of action by inhibiting the activity of COX and lipoxygenase enzymes and suppressing the formation of antigen-antibody complexes that occur in the glomerulus so that they can suppress inflammatory activation, free radical activity, and cytokine release. Flavonoids (quercetin and isoquercetin) protect cells from lipid peroxidation, which can inhibit the decrease in membrane permeability and cause cell necrosis (Anjani *et al.* 2018). The administration of ROPE has been shown to have the potential to repair damage to proximal tubular cells by inhibiting oxidative stress, which has a good impact on kidney biochemistry. Flavonoids can regenerate kidney function by reducing oxidative stress, which can reduce BUN and creatinine levels (Michael 2013). Therefore, the red okra pod has the potential as an affordable drug and natural antioxidant source to protect the proximal renal tubule from MNU toxicity.

Acknowledgements

This work was funded by Penelitian Unggulan Fakultas, Universitas Airlangga, Indonesia (Grant no. 251/UN3/2022).

References

Abou Zaid, O.A.R., Ahmed, F.A., Badwi, A.F.M., Ibrahim, N.M.H., 2017. Effect of natural nanoparticles products on hepato-renal functions in breast carcinoma female rats. *Benha Veterinary Medical Journal.* 33, 99-106. https://doi.org/10.21608/bvmj. 2017.30003

Adefisan, A., Owumi, S., Adaramoye, O., 2018. Root bark extract of *Calliandra portoricensis* (Jacq) Benth. chemoprevention N-Methyl-N-Nitrosourea induced mammary gland toxicity in rats. *Journal of Ethnopharmacology*. 233, 22-33. https://doi.org/10.1016/j.jep.2018.12.027

Ahn-Jarvis, J.H., Parihar, A., Doseff, A.I., 2019. Dietary flavonoids for immunoregulation and cancer: food design for targeting disease. *Antioxidants*. 8, 202. https://doi.org/10.3390%2Fantiox8070202

Akhlaghi M., Bandy B., 2009. Mechanism of flavonoid protection against myocardial ischemia-reperfusion injury. *Journal Molecullar and Cellular Cardiology*. 46, 309-317. http://doi.org/10.1016/j.yjmcc.2008.12.003

Anjani, P.P., Damayanthi, E., Rimbawan, R., Handharyani, E., 2018. Potential of okra (*Abelmoschus esculentus* L.) extract to reduce blood glucose and malondialdehyde (MDA) liver in streptozotocin-induced diabetic rats. *Indonesian Journal of Nutrition and Food.* 13, 47-54. https://doi.org/10.25182/jgp.2018.13.1.47-54

Ansori, A.N.M., Susilo, R.J.K., Hayaza, S., Winarni, D., Husen, S.A., 2019. Renoprotection by *Garcinia mangostana* L. pericarp extract in streptozotocin-induced diabetic mice. *Iraqi Journal of Veterinary Sciences.* 33, 13-19.

Carew, J.S., Zhou, Y., Albitar, M., Carew, J.D., Keating, M.J., Huang, P., 2003. Mitochondrial DNA mutation in primary leukemia cells after chemotherapy: clinical significance and therapeutic implications. *Leukemia.* 17, 1437-1447. https://doi.org/ 10.1038/sj.leu.2403043

Chaemsawang, W., Prasongchean, W., Papadopoulos, K. I., Ritthidej, G., Sukrong, S., Wattanaarsakit, P., 2019. The effect of okra (*Abelmoschus esculentus* (L.) Moench) seed extract on human cancer cell lines delivered in its native form and loaded in polymeric micelles. *International Journal of Biomaterials*. 2019, 9404383. https://doi.org/10.1155/2019/9404383

Chevalier, R.L., 2016. The proximal tubule is the primary target of injury and progression of kidney disease: role of the glomerulo-tubular junction. *American Journal of Physiology-Renal Physiology*. 311, F145-F161. https://doi.org/10.1152/ ajprenal. 00164.2016

Curthoys, N.P., Moe, O.W., 2014. Proximal tubule function and response to acidosis. *Clinical Journal of the American Society of Nephrology*. 9, 1627-1638. https://doi.org/10.2215%2FCJN.10391012

Faustino-Rocha, A.L, Ferreira, R., Oliveira, P.A., Gama, A., Ginja, M., 2015. *N*-Methyl-*N*-Nitrosourea as a mammary carcinogenic agent. *Tumour Biology*. 36, 9095-9117. https://doi.org/10.1007/s13277-015-3973-2

Fevrier-Paul, A., Soyibo, A.K, Mitchell, S., Voutchkov, M., 2018. Role of toxic elements in chronic kidney disease. *Journal of Health and Pollution.* 8, 181202. https://doi.org/10.5696%2F2156-9614-8.20.181202

Drabik-Markiewicz, G., Van den Maagdenberg, K., De Mey, E., Deprez, S., Kowalska, T., Paelinck, H., 2009. Role of proline and hydroxyproline in *N*-Nitrosamine formation during heating in cured meat. *Meat Science*. 81, 479–486. https://doi.org/10.1016/j.meatsci.2008.10.002

Gagné, F., 2014. Chapter 6-oxidative stress, in: Gagné, F. (Eds.), *Biochemical Ecotoxicology Principles and Methods*. Academic Press, Oxford, pp. 103–115. https://doi.org/10.1016/B978-0-12-411604-7.00006-4

Gal, A.F., Stan, L., Tăbăran, F., Rugină, D., Cătoi, A.F., Andrei, S., 2020. Chemopreventive effects of propolis in the MNU-induced rat mammary tumor model. *Oxidative Medicine and Cellular Longevity*. 2020, 4014838. https://doi.org/10.1155/ 2020/4014838

Griffin, B.R., Faubel, S., Edelstein, C.L., 2019. Biomarkers of drug-induced kidney toxicity. *Therapeutic Drug Monitoring*. 41, 213–226. https://doi.org/10.1097/FTD.0000000000000589

Husen, S., Winarni, D., Ansori, A., Susilo, R., Hayaza, S., Darmanto, W., 2020. Antioxidant potency of okra (*Abelmoschus esculentus* L. Moench) pods extract to ameliorate kidney structure and function in diabetic mice. *Ecology, Environment and Conservation*, 26, 1768–1774.

Kim, H.J., Kim, T.E., Han, M., Yi, Y., Jeong, J.C., Chin, H.J., Song, S.H., Lee, J., Lee, K.B., Sung, S., Han, S.H., Seong, E.Y., Ahn, C., Oh, K.H., Chae, D.W., 2021. Effects of blood urea nitrogen independent of the estimated glomerular filtration rate on the development of anemia in non-dialysis chronic kidney disease: The results of the KNOW-CKD study. *PloS One*. 16, e0257305. https://doi.org/10.1371/journal. pone.0257305

Koźmiński, P., Halik, P.K., Chesori, R., Gniazdowska, E., 2020. Overview of dual-acting drug methotrexate in different neurological diseases, autoimmune pathologies and cancers. *International Journal of Molecular Sciences*. 21, 1-38. https://doi.org/10.3390/ijms21103483

Liu, G.D., Hou, G.Y., Wang, D.J., Lv, S.J, Zhang, X.Y., Sun, W.P., Yang, Y., 2008. Skin pigmentation evaluation in broilers fed different levels of natural okra and synthetic pigments. *Journal of Applied Poultry Research.* 17, 498–504. https://doi.org/10.3382/japr.2008-00058

Luiking, Y.C., Engelen, M.P.K.J., Deutz, N.E.P., 2010. Regulation of nitric oxide production in health and disease. *Current Opinion in Clinical Nutrition and Metabolic Care*. 13, 97–104. https://doi.org/10.1097/MCO.0b013e328332f99d

Majd, E.N., Azizian, H., Tabandeh, M.R., Shahriari, A., 2019. Effect of *Abelmoschus esculentus* powder on ovarian histology, expression of apoptotic genes and oxidative stress in diabetic rats fed with high fat diet. *Iranian Journal of Pharmaceutical Research*. 18, 369–382.

Michael, M., 2013. Pengaruh ekstrak metanol daun kesum (*Polygonum minus* Huds.) terhadap peningkatan kadar kreatinin dan ureum serum tikus putih galur wistar terinduksi sisplatin. *Jurnal Mahasiswa Farmasi Fakultas Kedokteran UNTAN*. 1, 1-12.

Moon, K.Y., 2010. *N*-Nitroso-*N*-Methylurea and *N*-Nitroso-*N*-Ethylurea induce upregulation of cellular NF-kappa B activity through protein kinase C-dependent pathway in human malignant keratinocytes. *Archives of Pharmacal Research*. 33, 133–139. https://doi.org/10.1007/s12272-010-2235-5

Nijveldt, R.J., van Nood, E., van Hoorn, D.E., Boelens, P.G., van Norren, K., van Leeuwen, P.A., 2001. Flavonoids: a review of probable mechanisms of action and potential applications. *American Journal of Clinical Nutrition.* 74, 418-425. http://doi.org/10.1093/ajcn/74.4.418

Okada, H., Senmaru, T., Fukui, M., Kondo, Y., Ishigami, A., Maruyama, N., Obayashi, H., Yamazaki, M., Nakamura, N., Hasegawa, G., 2015. Senescence marker protein-30/gluconolactonase deficiency exacerbates diabetic nephropathy through tubular injury in a mouse model of type 1 diabetes. *Journal of Diabetes Investigation.* 6, 35-43. http://doi.org/10.1111/jdi.12252

Palm, M., Lundblad, A., 2005. Creatinine concentration in plasma from dog, rat, and mouse: a comparison of 3 different methods. *Veterinary Clinical Pathology*. 34, 232–236. https://doi.org/https://doi.org/10.1111/j.1939-165X.2005.tb00046.x

Paravicini, T.M., Touyz, R.M., 2008. NADPH oxidases, reactive oxygen species, and hypertension: clinical implications and therapeutic possibilities. *Diabetes Care*. 31, 170-80. https://doi.org/10.2337/dc08-s247

Pizzino, G., Irrera, R., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Domenica Altavilla, D., Bitto, A., 2017. Oxidative stress: harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*. 2017, 8416763. http://doi.org/10.1155/2017/8416763

Qiu, J., Yang, X., Wang, L., Zhang, Q., Ma, W., Huang, Z., Bao, Y., Zhong, L., Sun, H., Ding, F., 2019. Isoquercitrin promotes peripheral nerve regeneration through inhibiting oxidative stress following sciatic crush injury in mice. *Annals of Translational Medicine*. 7, 680. https://doi.org/10.21037/atm.2019.11.18

Rasool, M., Farooq, S., Arif Malik, A., Shaukat, A., Manan, A., Asif, M., Sani, S., Mahmood Husain Qazi, M.H., Kamal, M.A., Iqbal, Z., Hussain, A., 2015. Assessment of circulating biochemical markers and antioxidative status in acute *lymphoblastic leukemia* (ALL) and acute myeloid leukemia (AML) patients. *Saudi Journal of Biological Science*. 22, 106–111. http://doi.org/10.1016/j.sjbs.2014.09.002

Scott, J.A., Maarsingh, H., Holguin, F., Grasemann, H., 2021. Arginine therapy for lung diseases. *Frontiers in Pharmacology*. 12, 627503. https://doi.org/10.3389/fphar. 2021.627503

Syam, A., Riyanti, S., Widya, U., 2020. Kadar flavonoid dan polifenol buah okra merah dan okra hijau (*Albelmoschus esculentus* (L.) Moench). *In: Seminar Nasional Farmasi (SNIFA) 4, UNJANI*. Bandung: Universitas Jenderal Achmad Yani. pp. 5-8.

Tian, H., Cronstein, B.N., 2007. Understanding the mechanisms of action of methotrexate: implications for the treatment of rheumatoid arthritis. *Bulletin of the NYU Hospital for Joint Diseases*. 65, 168–173.

Tsubura, A., Yoshizawa, K., Sasaki, T., 2014. Methylnitrosourea. In: Wexler P (Eds.). *Encyclopedia of Toxicology, 3rd edition*. Oxford: Academic Press. pp. 321–323.

Tsuruma, K., Yamauchi, M., Inokuchi, Y., Sugitani, S., Shimazawa, M., Hara, H., 2012. Role of oxidative stress in retinal photoreceptor cell death in *N*-methyl-*N*-nitrosourea–treated mice. *Journal of Pharmacological Sciences.* 118, 351–362. http://doi.org/10.1254/jphs.11110FP

Ullah, A., Munir, S., Badshah, S.L., Khan, N., Ghani, L., Poulson, B.G., Emwas, A.H., Jaremko, M., 2020. Important flavonoids and their role as a therapeutic agent. *Molecules*. 25, 1-39. https://doi.org/10.3390/molecules25225243

Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M., Mazur, M., 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions*. 160, 1–40. https://doi.org/https://doi.org/10.1016/j.cbi.2005.12.009

Wahyuningsih, S.P.A., Mwendolwa, A.A., Winarni, D., Anggreini, R.W., Mamuaya, B.K. K., 2021. Protective effect of red okra (*Abelmoschus esculentus* (L.) Moench) pods against sodium nitrite-induced liver injury in mice. *Veterinary Medicine International.* 2021, 6647800. https://doi.org/10.1155/2021/6647800

Wahyuningsih, S.P.A., Savira, N.I.I., Anggraini, D.W., Winarni, D., Suhargo, L., Kusuma, B.W.A., Nindyasari, F., Setianingsih, N., Mwendolwa, A.A., 2020. Antioxidant and nephroprotective effects of okra pods extract (*Abelmoschus esculentus* L.) against lead acetate-induced toxicity in mice. *Scientifica.* 2020, 4237205. https://doi.org/10.1155/ 2020/4237205

Xia, F., Zhong, Y., Li, M., Chang, Q., Liao, Y., Liu, X., Pan, R., 2015. Antioxidant and anti-fatigue constituents of okra. *Nutrients*. 7, 8846–8858. https://doi.org/10.3390/nu7105435

Xue, Y., Daniels, L.B., Maisel, A.S., Iqbal, N., 2014. *Cardiac Biomarkers*. Elsevier, New York. https://doi.org/10.1016/B978-0-12-801238-3.00022-2

Zainuddin, A., Sabilu, Y., Majid, R., Pratiwi, A., Jafriati, 2022. Analysis of phytochemical compounds, total phenolic content, and antioxidant activity test of ethanol extract of okra (*Abelmoschus esculentus* L.) from the traditional market of Kendari. *Journal of Hunan University Natural Sciences.* 49, 89-95. https://doi.org/10.55463/issn.1674-2974.49.7.10