Amelioration Effect Of Eggplant Skin Ethanol Extract In Afb1 Nephrotoxis Male Rats On Changes In Serum Kim1, Ngal, Ureum, Creatinine, And Cystatin-C

Mutia Sari[1]., Gusbakti Rusip[2]., Fiska Maya Wardhani[3].Ade Indra,M[4]

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Abstract

Mycotoxin contamination in food, feed, and agricultural products is emerging as a severe problem because these toxic substances can cause various types of poisoning and, consequently, various health problems, from acute and chronic problems to causing animal and human death. Aflatoxin is a mycotoxin produced by several Aspergillus species, especially Aspergillus parasiticus and Aspergillus flavus, related to agricultural commodities cultivated in tropical and subtropical regions. Apart from interfering with protein synthesis, this toxin is carcinogenic, mutagenic, hepatotoxic, and nephrotoxic. Oxidative stress is an important molecular mechanism for kidney injury in aflatoxin B1 (AFB1) nephrotoxicity. Erythroid nuclear factor 2-related factor 2 (Nrf2) is a crucial transcription factor for regulating cellular oxidative stress responses, which has been confirmed in animal models. Antioxidants are used as food additives to protect against oxidative degradation and are also known to inhibit mold growth and the production of mycotoxins associated with several toxigenic Fusarium species. Eggplant is a vegetable commodity with anti-inflammatory, antioxidant, anti-viral, anti-bacterial, anti-toxic, and anti-cancer properties. Researchers evaluated the chemoprotective effect of ESEE (Eggplant skin ethanol extract) on AFB1-induced kidney injury (1 mg/kgBW); 50 male Wistar rats were randomly divided into ten groups and tested ESEE with doses of 200, 400, and 600 mg/kgBW. Experimental animals were evaluated for 28 days. Researchers found that exposure to AFB1 significantly increased serum concentrations of BUN (Blood urea nitrogen), creatinine, NGAL, Kim-1, and Cystatin-C and caused damage to the histological structure of the kidney. ESEE can potentially ameliorate AFB1-induced kidney lesions through attenuating AFB1 oxidative stress.

Keywords: Eggplant skin ethanol extract, Ureum, Creatinin, NGAL, Kim-1, Cystatin-C

[1]Faculty of Medicine, University Prima of Indonesia, [2]Department Physiology , Faculty of Medicine University Prima of Indonesia, [3]Department Histology, Faculty of Medicine University Prima of Indonesia. [4] Deparment Surgery, Faculty of Medicine University Prima of Indonesia.

Correspondent author**:** Gusbakti Rusip

Introduction

Mycotoxins are natural contaminants in food and agricultural products worldwide. These secondary metabolites are produced by toxigenic fungi, classified as fungal species that can produce one or more mycotoxins. Several species of fungi can produce mycotoxins; for example, aflatoxins and ochratoxins are produced by more than one fungus species, contributing to the year-round presence of mycotoxins (Omatayo *et al.*, 2018). Exposure of mycotoxins to food, feed, and agricultural products has emerged as a matter of grave concern, as these toxic substances can cause various types of poisoning and, consequently, a wide range of health problems, from acute and chronic problems to death in animals and humans. Based on data from the Directorate of Environmental Health and the Public Health Emergency Operation Center (PHEOC) of the Ministry of Health (Kemenkes), in 2017, there were 163 extraordinary food poisoning incidents recorded, with a Case Fatality Rate (CFR) of 0.1% (Saefulm,2020, Ade Indra et al.,2021).

Mycotoxin toxicity impacts the human cellular genome. Along with the importance of mycotoxin toxicity and toxicological mechanisms, there are associated health problems arising from exposure to these toxins, including DNA damage, kidney damage, DNA/RNA mutations, growth retardation in children, gene modification, and immune disorders. Mycotoxins are toxic metabolic compounds produced by molds. Many mycotoxins have chemical stability and can withstand the rigors encountered throughout the food supply chain. The most common mycotoxins of concern to humans and livestock include aflatoxins, citrinin, ochratoxins, fumonisin, patulin, zearalenone, nivalenol, deoxynivalenol, fumonisin, and ergot alkaloids (Awuchi *et al.*, 2022). The world health organization (WHO) has classified some of these as human carcinogens. Most of the mycotoxins known today are classified according to their toxic activity in chronic conditions into mutagenic, carcinogenic, or teratogenic mycotoxins. For example, naturally occurring aflatoxins are classified as human carcinogens (Group 1); ochratoxins and fumonisin are classified as probable human carcinogens (Group 2B), whereas trichothecenes and zearalenone are not recognized as human carcinogens (Group 3). However, almost all mycotoxins can cause one or more significant health problems. In addition, some of these mycotoxins suppress the immune system, exposing consumers to health threats (Omatayo *et al.*, 2018).

Aflatoxin B1(AFB1) is a secondary metabolite produced by the ubiquitous Aspergillus fungus, such as moldy grain products, and has been reported to cause hepatotoxicity, nephrotoxicity, and reproductive toxicity (Zhang *et al.*, 2022). This toxin can interfere with protein synthesis, is carcinogenic, mutagenic, inhibit cleaning of lung particles, damages the macrophage system, increases sensitivity to bacterial endotoxins, stunts children's growth, and reduces body resistance (Awuchi *et al.*, 2022), heart and kidney muscle injury (Yilmaz *et al.*, 2018). In 2015 the World Health Organization (WHO) reported its first global and regional disease burden estimates due to 31 food contamination hazards. Research results show that every year, 1 in 10 people get sick from food contaminated with microbes or chemicals, resulting in 600 million illnesses, 420,000 deaths, and a loss of 33 million years of healthy life globally (Pires *et al.*, 2021). Antioxidants have been used as food additives to protect against oxidative degradation and are also known to inhibit mold growth and mycotoxin production associated with several toxigenic Fusarium species. Several natural (vitamins, provitamins, carotenoids, polyphenols, and micronutrients) and synthetic compounds appear to have chemo-protective effects against common mycotoxins (Neff *et al.*, 2018). Eggplant, known by the scientific name Solanum melongena L., is a popular vegetable commodity liked by many people and is widely used as a traditional medicine in China with anti-inflammatory, antioxidant, anti-viral, anti-bacterial, and anti-cancer properties. Amides and phenylpropanoids are the two main constituents in eggplant. Four amides that have therapeutic effects and anticytotoxic activity in eggplant, including:(1)N-trans-p-coumaroyltyramine,(2)N-trans-p-coumaroyloctopamine,(3)N-trans-p-coumaroyl noradrenline, and (4)N-trans -feruloyl octopamine and phenylpropanoid neochlorogenic acid

Amide and phenylpropanoid metabolism is mainly involved in hydroxylation, methylation,

glucuronidation, or sulfation reactions to explain the primary pharmacodynamic mechanism of metabolites 1-5 identified in eggplant that CYP-450 enzymes can hydroxylate. Lelario *et al.* (2019) argue that the content of flavonoids, alkaloids (amides and glycoalkaloids), phenolic acids, and steroids are the primary and essential chemical components in eggplant which are very helpful in the treatment of cancer diseases such as liver cancer, cervical cancer, breast cancer, and other diseases. Bowen inhibits the development of the cell cycle in the "S" phase, induces progressive cell apoptosis, and inhibits the development of pathogens. Li *et al.* (2018) have proven that the content of N-trans-coumaroyltyramin, n-trans-feruloyl tyramine, and n-trans-feruloyl octopamine in eggplant can show effective radical scavenging activity. Zielinski *et al.* (2020) proved that eggplant's neochlorogenic acid phenylpropanoid compound has antioxidant activity using ABTS, DPPH, and FRAP (Song *et al.*, 2021).

This experimental method to determine the effect or relationship of the independent variable with the dependent variable (nephroprotective activity). The independent variable is ESEE, while the dependent variable is a decrease in values: (1) screening for kidney problems (plasma urea and creatinine), (2) assessing Cystatin-C kidney function, (3) assessing glomerular function: NGAL, (4) assessing KIM-1 proximal renal tubule injury. (5) perform a histological examination of the kidneys with HE (Hematoxylin-eosin) staining.

Material AND Methode

1. Animal and experimental design.

Fifty adult male Wistar rats (2-3 months, ± 200 g) were housed in the Pharmacology Laboratory, Faculty of Pharmacy, University of North Sumatra, Medan, Indonesia. Mice were maintained in polypropylene cages in well-ventilated rooms at 24 ± 10 C and 12 h light/dark cycle. All rats were given a husk diet and a 0.5 % Na-CMC drink. After one week of acclimatization, the rats were divided into ten groups. Group 1 (Neutral), only given 0.5% Na-CMC feed and drink, the following nine groups were induced by AFB1 in a single dose of 1 mg/kg BW/IM with different treatment, namely in group 2 (control group (-) = AFB1 group) induced only, group 3 (control group +1) was given Vitamin C 1.62 mg day 5 to day 28. Group 4 (control group +2) was given Vitamin C 1.62 mg from day 1 to day 28. Group 5 (TG=treatment group 1) was given an ESEE dose of 200 mg/kg BW from day 5 to day 28. Group 6 (TG 2) was given the same dose of ESEE from day 1 to day 28. Group 7 (TG 3) was given ESEE 400 mg/kg BW from day five until day 28. Group 8 (TG 4) was given an ESEE dose of 400 mg/kgBW on the first day until day 28. Group 9 (TG 5) was given ESEE 600 mg/kgBW from day 5 to day 28. Group 10 (TG 6) was given ESEE 600 mg/kgBB from the first day until day 28. The purpose of giving vitamin C and ESEE on the first day was to test for amelioration. In contrast, the group was given vitamin C and ESEE starting from day five to test the effectiveness of therapy. The rats' health status was monitored daily during the study period, and the rat mortality rate was 0. The use of the rats and the experimental protocol were approved by the Ethics Clearance Committee of the Faculty of Medicine, Universitas Prima Indonesia.

2. Sample collection

After 28 days of monitoring, three rats were randomly taken from each group; rats were terminated after intravenous. Ketamine anesthesia 70 mg/kg.bw. Blood was collected through the heart, and 3 mL of each group was taken with a syringe, then put into a non-EDTA tube and centrifuged for 10 minutes at 3000-4000 rpm to produce two layers, namely serum/supernatant and precipitate. The serum layer was then taken using a 1 mL syringe, accommodated in a microtube, and stored in the refrigerator at -4°C (Hubrecht, R.J.,2010, gusbakti et al.,2022).

3. Analysis of BUN, Creatinine, NGAL, Kim-1, and Cystatin-C

The concentrations of BUN, Creatinine, NGAL, Kim-1, and Cystatin-C levels were determined using a blood spectrophotometer at a wavelength of 450 nm.

4. Histopathological analysis

Kidney tissue samples were collected by performing a biopsy to determine the degree of tissue damage base was thick and stained with hematoxylin and eosin (H&E). The kidney tissue was embedded in paraffin, section to a 4 um. The stain section was the exam under a light microscope (200x magnification) with ten fields of view to determine the degree of

damaged tissue. (Leake, R., et al. 2000, Gusbakti et al., 2022)

Statistical analysis

Data are expressed as mean ± SD (standard deviation). Statistical significance was analyzed using Way ANOVA followed by the LSD test as a post hoc test. For the results of the comparison of all groups with the statement, P> 0.05 was significantly the same, and P < 0.05 was not significantly the same. Analysis was performed with SPSS software version 22.

Results

1.NGAL, Creatinin, BUN, Kim-1, and Cystatin results.

The research results can be seen in table 1 below:

Table 1 Test results for NGAL, Creatinin, BUN, kim-1, and Cystatin-C

No.	Groups	NGAL (ng/mL) Mean ± **SD**	Creatinine (mg/dL) **Mean ± SD**	BUN(mg/dL) **Mean ± SD**	Kim-1(ng/mL) **Mean ± SD**	Cystatin-C (mg/dL) **Mean ± SD**
1.	Netral	52.63±0.75	0.26±0.13	11.55±1.42	26.99±.0.032	0.68 ±.0.03
2.	Control (-)	158.63±1.51	9.47±0.71	74.68±7.05	161.48±0.010	4.93±0.020
3.	Control (+1)	61.27±1.29	2.01±0.02	23.09±6.60	50.79±0.010	2.65±0.030
4.	Control (+2)	51.97±1.76	0.92±0.05	16.79±3.26	38.29±.0.021	1.04±.0.030
5.	T-Group 1	92.42±2.22	4.81±0.38	57.73±7.90	68.69±.0.020	2.69±.0.025
6.	T-Group 2	80.35±2.11	4.18±0.18	54.15±4.31	60.54±0.020	2.48±0.035
7.	T-Group 3	71.46±1.28	2.56±0.27	46.79±7.72	50.89±0.020	2.4±0.030
8.	T-Group 4	62.47±1.07	1.97±0.04	40.60±6.81	46.31±0.021	1.19±0.020
9.	T-Group 5	57.39±1.01	1.0±0.07	19.61±4.93	32.15±0.010	1.01±0.025
10.	T-Group 6	52.16±1.01	0.39±0.12	16.69±5.85	25.81±0.015	0.69±0.035

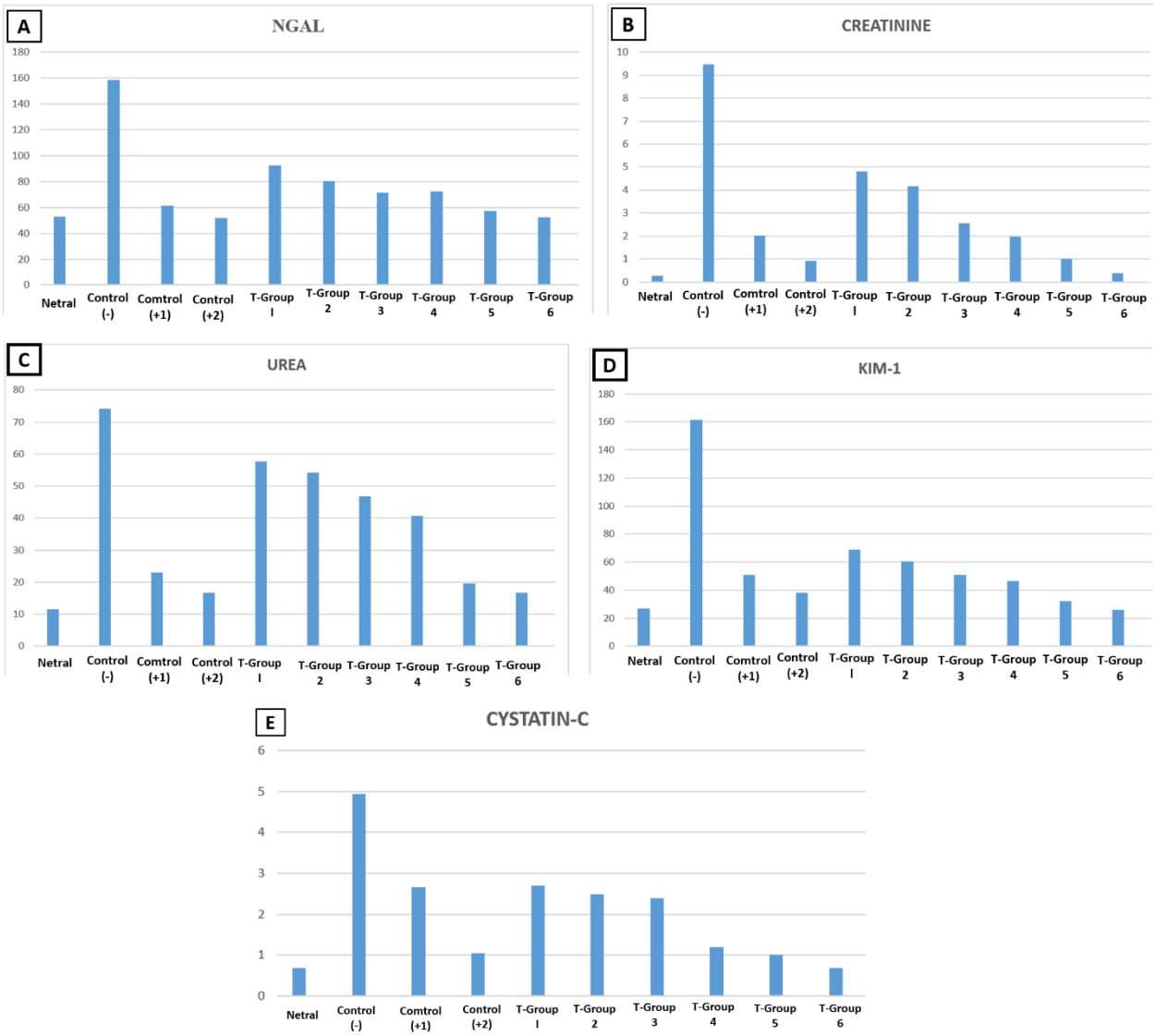
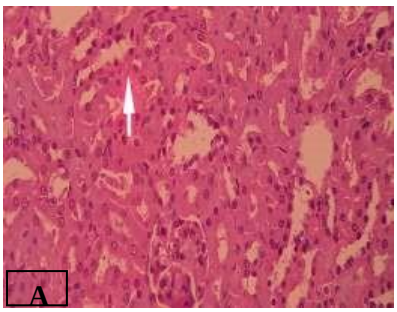


Figure 1. The protective effects of ESEE in kidney damage caused by AFB1. (A) Content levels of NGAL, (B) Content levels of Creatinine, (C) Content levels of BUN, (D) Content levels of Kim-1, (E) Content levels of Cystatin-C.

1.1 In the "Oneway" descriptive analysis test, the worst values for NGAL, Creatinine, BUN, Kim-1, and Cystatin-C are in the negative group. The best values are test-6 and positive control 2. Compared to the negative control group, all ESEE and vitamin C doses reduced these levels ($P<0.05$).

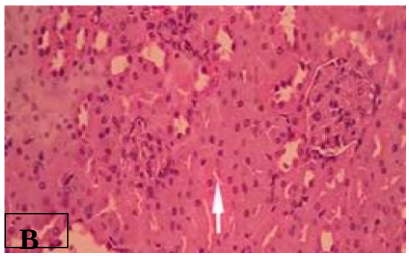
1.2 Histopathological results

1. Neutral Group

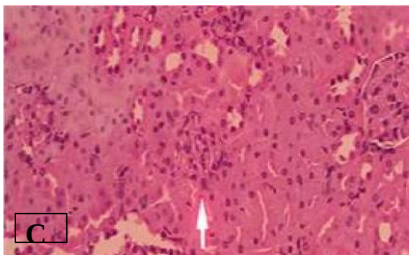


Neutral (No damage occur)

1. Negatif Control Group 1

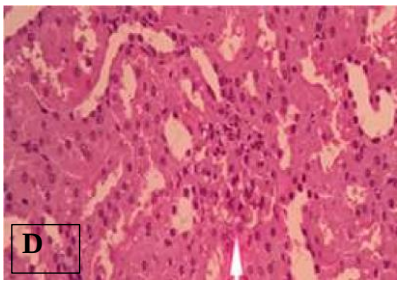


Gambar B
Degenerated cells, inflammatory cell infiltration,



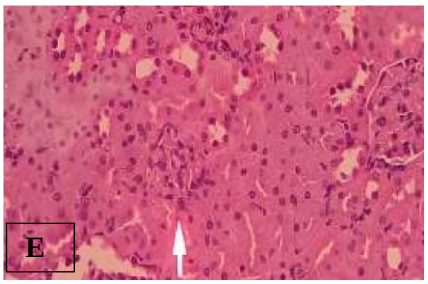
Gambar C
, Degenerated cells, Necrosis, glomerular damage

2. Positif Control Group 1



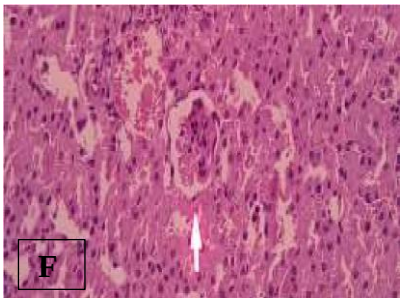
Gambar D

Glomerular congestion, tubular lumen damage



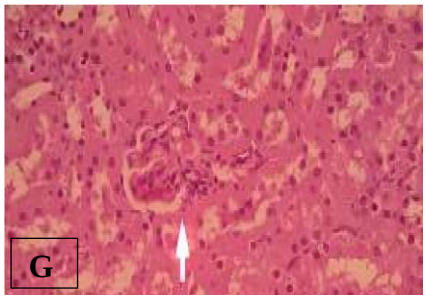
Gambar E
Degenerasi swelling, Nekrosis

3. Positif Control Group 2



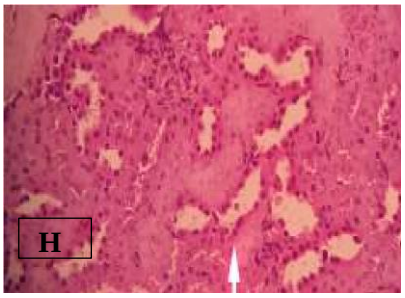
Gambar F

Glomerular damage, Pigment deposits



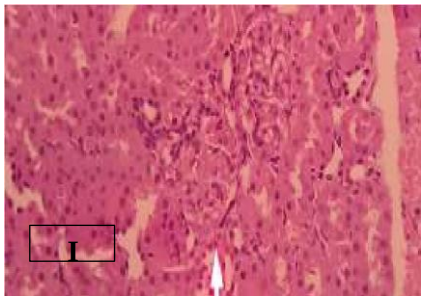
Gambar G
Degenerated cells

4. Kelompok Perlakuan I



Gambar G

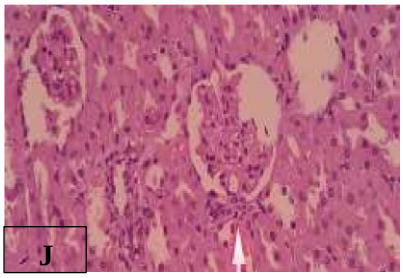
Sel yang mengalami degenerasi, kerusakan lumen



Gambar I

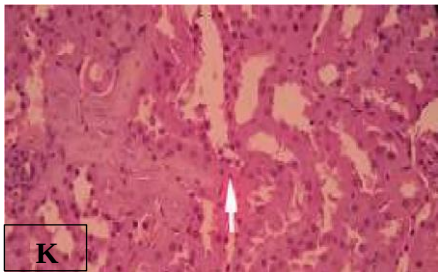
Celah bomen yang menyempit, Nekrosis

5. Kelompok Perlakuan 2



Gambar J

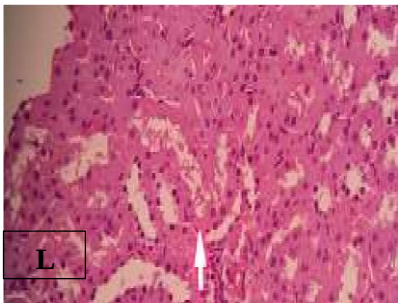
Degenerasi swelling, Hemoragi



Gambar K

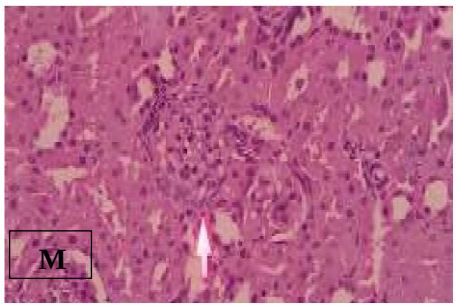
Kerusakan lumen tubulus, deposit pigment

6. Kelompok Perlakuan 3



Gambar L

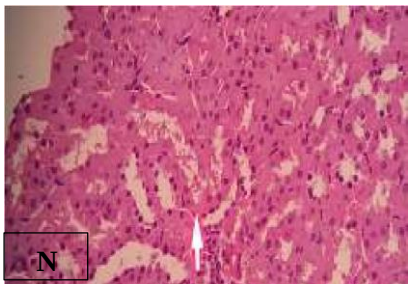
Kerusakan lumen tubulus, deposit pigment



Gambar M

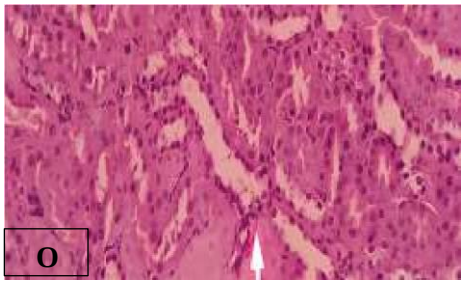
Tubulus Kontortus Proksimal, sel yang mengalami degenerasi

7. Kelompok Perlakuan 4



Gambar N

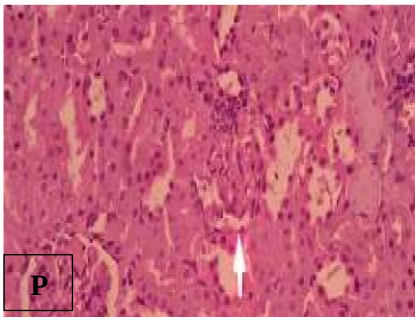
Celah bomen yang menyempit, nekrosis



Gambar O

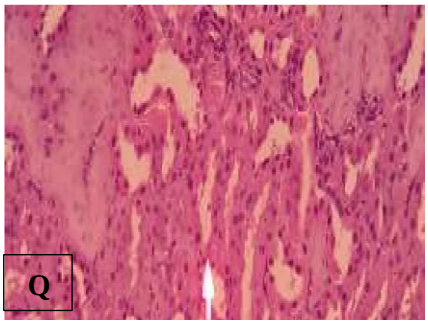
Sel yang mengalami degenerasi

8. Kelompok Perlakuan 5



Gambar P

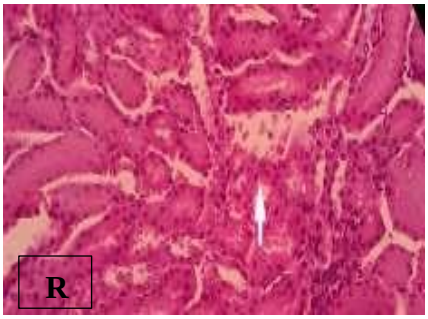
Deposit Pigment, kongesti glomelurus



Gambar O

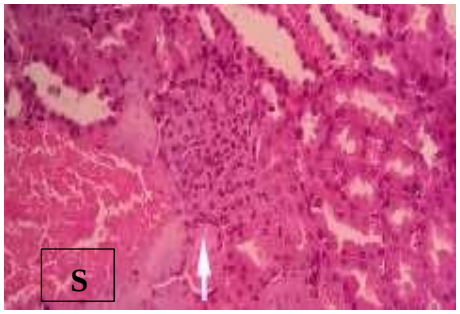
Deposit Pigment, nekrosis epitel tubulus proksimal

9. Kelompok Perlakuan 6



Gambar R

Deposit Pigment



Gambar S

Sel yang mengalami

Figure 2. Pathological kidney tissue detection by pathological staining with hematoxylin and eosin (H&E), the picture were captured at 200x magnification, in each groups.

Tabel 2 Renal histopathologi

No.	Groups	Marker	Necrosis level (%)
1.	(Netral)	A	0
2.	(kontrol neg-1)	B	60
3.	(kontrol pos+1)	C	10
4.	(kontrol pos+2)	D	10
5.	(Treatment G-1)	E	35
6.	(Treatment G-2)	F	30
7.	(Treatment G-3)	G	20
8.	(Treatment G-4)	H	25
9.	(Treatment G-5)	I	13
10.	(Treatment G-6)	J	11

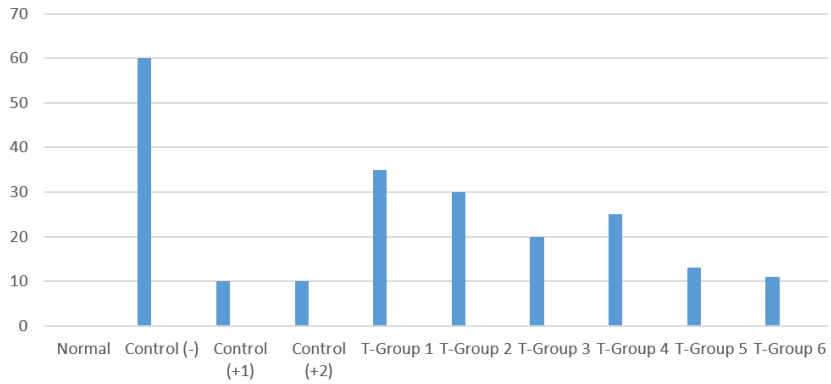


Figure 3. The pathological pictures in each group were analyzed through scoring system scanning, and the scanning score was represented as %.

The neutral group is a normal histopathological appearance in rat kidneys, while the negative group is a very severe histopathological picture. At a dose of 600 mg/kg.bw, the ESEE almost resembled the histopathological picture in the vitamin-C-positive control group.

Discussion

The US Food and Drug Administration considers AFB1 an unavoidable food contaminant. About 5 billion people in developing countries risk chronic exposure to AFB1. AFB1 can accumulate in the kidney and cause kidney injury, including renal inadequacy and disorganization. Oxidative stress is a significant risk factor for AFB1 nephrotoxicity. AFB1 exposure increases the presence of reactive oxygen species (ROS) and impairs the kidney's antioxidant defense system, resulting in renal oxidative damage. Oxidative stress is an important molecular mechanism for kidney injury in aflatoxin B1 (AFB1) nephrotoxicity. Erythroid nuclear factor 2-related factor 2 (Nrf2) is a crucial transcription factor for regulating cellular oxidative stress responses, which has been confirmed in animal models (Yu *et al.*, 2018). Oxidative stress is an important molecular mechanism for kidney injury in aflatoxin B1 (AFB1) nephrotoxicity. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a master transcription factor for regulating the cellular oxidative stress response, which has been confirmed in animal models *(Yu *et al.*, 2018). According to Wang *et*

al. (2022), because drug metabolites or toxic substances are selectively absorbed and concentrated by renal tubular cells before being excreted in the urine, high concentrations of toxic substances are also stored in the renal medulla. This suggests that the kidney is also involved in the critical accumulation of AFB1 toxin and increases the content of reactive oxygen species (ROS), which can impair cellular redox homeostasis, leading to kidney injury due to oxidative stress. In addition to inducing renal oxidative stress and disrupting the Keap 1-Nrf2 pathway and its downstream genes, as manifested by increased oxidative stress metabolites and decreased antioxidant enzyme activity, AFB1 also increased the percentage of apoptotic cells in the kidney via the Tunel test, along with increased expression of Cyst-c, Bax. , cleaved-Caspase-3, Caspase-9, and decreased Bcl-2 expression in transcription and protein levels; conversely, antioxidants can be relied upon to suppress oxidative stress mitigation and as an effective strategy to treat AFB1 nephrotoxicity (Wang *et al.*, 2022).

AFB1 is excreted via the kidney and can accumulate in the kidney, and eventually lead to kidney injury, including decreased renal glomerular filtration and tubular resorption, tubular cell vacuolation, interstitial nephritis, and glomerular swelling. This affects the increase in concentrations of BUN, creatinine, NGAL, Kim-1, Cystatin-C, and changes in histopathological kidneys and histopathological changes in our study indicate that AFB1 causes kidney damage. BUN, creatinine, NGAL, Kim-1, and Cystatin-C concentrations can be interpreted as indices of kidney dysfunction. Histopathological changes are indicators used to evaluate the damage to the structures of the kidney. Li *et al.* (2019) analyzed the toxicity and related mechanisms of AFB1 and AFM1 in the kidney using a 28-day toxicity rat model. Thirty rats were divided into six groups: control (no treatment), L-proline group (10 g/kg body weight (b.w.)), AFB1 group (0.5 mg/kg b.w.), AFM1 (3.5 mg/kg b.w.), the AFB1 + L-proline group, and the AFM1 + L-proline group. Renal indices and biochemical indicators were detected, and pathological staining was observed. Using the human embryonic kidney cell model 293 (HEK 293), AFB1 and AFM1 were found to be toxic to the mouse kidney, causing abnormal expression of biochemical indicators related to kidney function, as well as pathological staining of kidney tissue, and the degree of cell apoptosis and expression of apoptotic proteins was detected. The results showed that the AFB1 and AFM1 activation pathways are related to oxidative stress and lead to kidney injury. AFB1 and AFM1 have a similar chemical structure; AFM1 can be derived from AFB1 (4-hydroxy AFB1 derivative, Figure S1) in the liver by hepatic microsomal cytochrome P450, and can then enter the blood circulation and be excreted via lactation into milk [3]. The two aflatoxins are considered to be the most common and carcinogenic members of the AF family, and the International Agency for Research on Cancer (IARC) organization clearly suggested that AFB1 and AFM1 should be classified as Group I carcinogens. Li *et al.* have proven AFB1 and AFM1 to be toxic to rat kidney and causes abnormal expression of biochemical indicators related to kidney function, as well as pathological staining of kidney tissue (Li *et al.*, 2019). Song *et al.* (2021) stated that Solanum melongena Extract has significant beneficial effects in the treatment of several types of cancer, such as liver cancer, cervical carcinoma, breast cancer, and Bowen's disease. Solanum melongena extract can inhibit cell cycle progression in the S phase, which induces progressive cell apoptosis and, finally, cell death. The content of N-trans-coumaroyltyramine, n-trans-feruloyl tyramine, and n-trans-feruloyloctopamine shows effective radical scavenging activity. Zielinski *et al.* (2020) revealed the neochlorogenic acid content of phenylpropanoid compounds which are antioxidants in vitro, using three methods (ABTS, DPPH, and FRAP) (Song *et al.*, 2021). In this study, we investigated the protection of ESEE against AFB1-induced nephrotoxicity, and importantly, our data demonstrated that ESEE inhibited AFB1-induced increases in Urea, creatinine, NGAL, Kim-1, and Cystatin-C content, as well as cell degeneration and necrosis. Nephrocyte kidney. These results suggest that ESEE significantly inhibits AFB1-induced kidney injury. Therefore, ESEE could be a useful preventive and therapeutic drug to inhibit AFB1-induced nephrotoxicity. In addition, exposure to AFB1 may cause serious kidney damage, which may lead to impaired excretion of AFB1 metabolites and amplify

renal injury, but these views warrant further exploration.

Conclusion

Ethanol extract of eggplant skin doses of 200, 400, and 600 mg/kg,bw can affect and improve kidney problems and function in nephrotoxic rats due to AFB1 induction in invivo studies.

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