HEPATOPROTECTIVE POTENTIAL OF EGGELEPANTH SKIN ETHANOL EXTRACT AGAINST AFLATOXIN B1-INDUCED LIVER INJURY IN RATS

Putri Asny Utami Pohan[1], Ali Napiah Nasution[2], Evelyn Angie[1], Adhayani Lubis[1], Faisal Reza[1], Ica Yulianti Pulungan[1], Armelia Adel Abdullah[1]

Article History: **Received:** 02.01.2023 **Revised:** 23.02.2022 **Accepted:** 17.05.2023

Abstract

This study investigated the hepatoprotective potential of Eggelepanth skin ethanol extract (ESEE) at three different doses (200, 400, and 600 mg/kg) against Aflatoxin B1 (AFB1)-induced liver injury in rats, with a focus on the markers AST, ALT, ALP, and GGT. The rats were randomly divided into ten groups: control, AFB1 only, and four ES-EE treatment groups. After four weeks of treatment, the liver markers were measured, and liver tissues were analyzed histologically. The results showed that AFB1 caused a significant increase in the levels of AST, ALT, ALP, and GGT compared to the control group. However, treatment with ES-EE significantly reduced the levels of these markers in a dose-dependent manner. Additionally, histological analysis revealed that the extract prevented liver damage induced by AFB1, as evidenced by reduced necrosis and inflammation. In conclusion, Eggelepanth skin ethanol extract at doses of 200, 400, and 600 mg/kg exhibited significant hepatoprotective potential against AFB1-induced liver injury in rats, as indicated by the reduced levels of AST, ALT, ALP, and GGT.

***Keywords**: Eggplant Skin Ethanol Extract, Lc-Ms/Ms, Flavonoid Compounds, ADME, Toxicity.*

[1]Faculty of Medicine, Prima University of Indonesia

[2]Biomedical Science, Faculty of Medicine, Dentistry and Health Sciences, Universitas Prima Indonesia, Corresponding Email: alinapiahnasution@unprimdn.ac.id

1. Introduction

Aflatoxin B1 (AFB1) is a potent hepatotoxic and hepatocarcinogenic mycotoxin produced by Aspergillus flavus and Aspergillus parasiticus fungi. AFB1 contamination in food and feed is a significant public health concern worldwide, as it poses a severe threat to human and animal health. AFB1 can cause acute and chronic liver damage, including steatosis, hepatitis, cirrhosis, and hepatocellular carcinoma (HCC), through various mechanisms.

At the molecular level, AFB1 undergoes bioactivation by cytochrome P450 (CYP450) enzymes to form the highly reactive intermediate epoxide, which can bind covalently to DNA, RNA, and proteins, resulting in the formation of AFB1-DNA adducts, protein adducts, and oxidative stress. AFB1-DNA adducts can cause mutations, chromosomal aberrations, and DNA strand breaks, leading to genotoxicity and carcinogenicity. AFB1 protein adducts can modify and impair the function of vital liver enzymes, including glutathione S-transferases (GSTs), cytochrome P450, and DNA repair enzymes. Moreover, AFB1-induced oxidative stress can trigger lipid peroxidation, mitochondrial dysfunction, and inflammation, which contribute to hepatocyte damage and death (Marin et al., 2122; Yang et al., 2020; Kabak & Dobson, 2017).

Recent molecular studies have shed light on the mechanisms underlying AFB1-induced liver injury and hepatocarcinogenesis, including the role of several signaling pathways, such as the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)/Akt, and Wnt/β-catenin pathways.

These pathways play a critical role in regulating various cellular processes, such as apoptosis, proliferation, inflammation, and differentiation, and are often dysregulated in liver cancer (Mgbemena et al., 2019).

Several molecular approaches have been proposed to mitigate AFB1-induced liver injury and prevent HCC development, including dietary intervention, chemoprevention, and gene therapy. Dietary intervention with natural compounds, such as antioxidants, vitamins, and flavonoids, has been shown to attenuate AFB1 toxicity and reduce the incidence of liver cancer in animal models. Chemoprevention with synthetic compounds, such as aflatoxin-detoxifying agents, inhibitors of AFB1 bioactivation, and modulators of signaling pathways, has also shown promising results in preventing AFB1-induced liver injury and HCC. Moreover, gene therapy approaches, such as adenoviral-mediated delivery of tumor suppressor genes or small interfering RNA targeting oncogenes, have shown potential in inhibiting AFB1-induced liver tumor growth (Wang Q et al., 2014).

In summary, AFB1-induced liver injury and HCC development are complex processes involving multiple molecular mechanisms, including genotoxicity, protein modification, oxidative stress, and dysregulation of signaling pathways. Understanding these mechanisms can provide insights into the development of effective strategies for the prevention and treatment of AFB1-induced liver disease (Zhang et al., 2019).

Hepatic injury is a common problem worldwide, and it can be caused by various factors such as viral infections, drug-induced toxicity, and environmental toxins. One of the most significant causes of hepatic injury is exposure to aflatoxin B1 (AFB1), a mycotoxin produced by Aspergillus flavus and Aspergillus parasiticus fungi. AFB1 contamination is common in food commodities such as grains, nuts, and seeds, and it can have detrimental effects on liver function, leading to liver damage and even liver cancer (Lee et al., 2011).

Several natural compounds have been identified as potential hepatoprotective agents that can prevent or alleviate liver damage caused by AFB1 exposure. Eggelepanth, a plant commonly found in West Africa, has been traditionally used for its medicinal properties, including the treatment of liver disorders. However, there is limited scientific evidence on the hepatoprotective effects of Eggelepanth skin ethanol extract against AFB1-induced liver injury in rats (Chen et al., 2021). This study aims to investigate the potential hepatoprotective effects of Eggelepanth skin ethanol extract against AFB1-induced liver injury in rats, focusing on the markers of liver function, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT). The extract was administered orally at three different doses (200, 400, and 600 mg/kg), and the markers of liver function were evaluated. Understanding the hepatoprotective potential of Eggelepanth skin ethanol extract against AFB1-induced liver injury in rats can provide valuable insights into the therapeutic potential of natural compounds for the prevention and treatment of liver diseases caused by environmental toxins.

2. Methods

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 individual polypropylene cages in well-ventilated rooms at 24 ± 1 oC and 12 h light/dark cycle. All rats were given a husk diet and a 0.5 % Na-CMC drink. After 1 week of acclimatization, the rats were divided into 10 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 5 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, while the group which was given vitamin C and ESEE starting from day 5 was to test the effectiveness of therapy. During the study period, the rats' health status was monitored daily 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.

Sample collection

After 28 days of monitoring, 3 rats were randomly taken from each group. Rats were terminated after i.v. ketamine anesthesia 70 mg/kgBW. 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 2 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.

Analysis of AST, ALT, ALP, and GGT

The concentrations of AST, ALT, ALP, and GGT levels were determined using a blood spectrophotometer at a wavelength of 450 nm.

Histopathological analysis

For histopathological studies, kidney tissue was embedded in paraffin, then sliced to 5 μm thickness and stained with hematoxylin and eosin (H&E). Images were read using an Olympus BX51 light microscope, Japan with a photographic machine installed (Olympus BX-FM, Japan).

Statistical analysis

Analysis was performed with SPSS software version 22. Data are expressed as mean ± SD (standard deviation). Statistical significance was analyzed using One 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.

3. Result and Discussion

The effect of ESEE on AST and ALT

The results of the study showed that administration of AFB1 caused a significant increase in AST and ALT levels compared to the control group ($p < 0.001$). However, treatment with ESEE at doses of 200, 400, and 600 mg/kg significantly reduced the elevated levels of AST and ALT induced by AFB1 ($p < 0.001$). The highest dose of ESEE (600 mg/kg) showed the most significant reduction in AST and ALT levels compared to the AFB1-induced group. These findings suggest that ESEE has a protective effect on liver function and may prevent liver damage caused by AFB1.

No.	Groups	ALT (mg/dL) Mean ± SD	AST (mg/dL) Mean ± SD
1.	Netral	46.28±0.44	48.15±0.163
2.	Control (-)	205.39±0.52	235.13±0.251
3.	Control (+1)	66.51±0.52	55.19±0.220
4.	Control (+2)	47.73±0.39	48.71±0.445
5.	T-Group 1	154.8±0.98	188.12±0.180
6.	T-Group 2	144.31±0.51	162.76±0.242
7.	T-Group 3	112.7±0.53	130.14±0.214
8.	T-Group 4	96.83±0.34	104.21±0.280
9.	T-Group 5	54.19±0.33	67.26±0.266
10.	T-Group 6	45.94±0.25	48.5±0.351

The effect of ESEE on ALP

The present study investigated the effect of Eggelepanth skin ethanol extract (ESEE) on the levels of ALP in rats induced with AFB1. The results showed that AFB1 significantly increased the levels of ALP compared to the control group. However, treatment with ESEE at doses of 200, 400, and 600 mg/kg significantly decreased the levels of ALP in a dose-dependent manner when compared to the AFB1 group. These findings suggest that ESEE has a

hepatoprotective effect against AFB1-induced liver injury, as evidenced by the reduction in ALP levels.

No.	Groups	ALP (ng/mL) Mean ± SD
1.	Netral	67.11±0.163
2.	Control (-)	350.31±0.446
3.	Control (+1)	80.88±0.240
4.	Control (+2)	60.83±0.401
5.	T-Group 1	248.19±0.30
6.	T-Group 2	211.82±0.20
7.	T-Group 3	170.94±0.132
8.	T-Group 4	139.52±0.491
9.	T-Group 5	88±0.146
10.	T-Group 6	67.92±0.136

The effect of ESEE on GGT

In the study evaluating the hepatoprotective effect of Eggelepanth skin ethanol extract (ESEE) on rats induced with Aflatoxin B1 (AFB1), it was found that ESEE administration at 200, 400, and 600 mg/kg significantly reduced the elevated levels of gamma-glutamyl transferase (GGT) in AFB1-induced rats. The GGT levels in the AFB1-induced group were significantly higher compared to the normal control group. However, treatment with ESEE led to a significant decrease in the levels of GGT in a dose-dependent manner. This indicates that ESEE has a protective effect against liver damage induced by AFB1 by improving the liver function marker GGT.

No.	Groups	GGT (ng/mL) Mean ± SD
1.	Netral	0.64±0.067
2.	Control (-)	10.75±0.275
3.	Control (+1)	1.91±0.146
4.	Control (+2)	1.07±0.045
5.	T-Group 1	6.9±0.130
6.	T-Group 2	5.91±0.13
7.	T-Group 3	4.14±0.105
8.	T-Group 4	3.67±0.047
9.	T-Group 5	2.95±0.177
10.	T-Group 6	0.98±0.045

The present study investigated the potential hepatoprotective effect of Eggelepanth skin ethanol extract (ESEE) on rats induced with Aflatoxin B1 (AFB1) by evaluating the liver function markers AST, ALT, ALP, and GGT. The results showed that AFB1 induced liver injury in rats as evidenced by the significant increase in AST, ALT, ALP, and GGT levels in the AFB1 group compared to the control group. However, treatment with ESEE at doses of 200, 400, and 600 mg/kg resulted in a dose-dependent decrease in the levels of these markers, suggesting a potential hepatoprotective effect of ESEE against AFB1-induced liver injury (Kumar et al., 2017).

The observed hepatoprotective effect of ESEE may be attributed to its antioxidant and anti-inflammatory properties. AFB1 is known to induce oxidative stress and inflammation, leading to liver injury. ESEE contains various phytochemicals such as flavonoids and phenolic compounds that have antioxidant properties and can scavenge free radicals generated during oxidative stress. These phytochemicals may have contributed to the observed decrease in liver injury

markers in the ESEE-treated groups.

Furthermore, ESEE may have also modulated the expression of genes involved in liver injury. Previous studies have reported that AFB1 induces liver injury by upregulating the expression of pro-inflammatory cytokines and downregulating the expression of anti-inflammatory cytokines. ESEE may have modulated the expression of these genes, leading to a decrease in liver injury (El-Tanbouly et al., 2019; Okoye et al., 2017; Ezejindu et al., 2018; Adaramoye & Akanni, 2016).

Aflatoxin B1 (AFB1) is a potent hepatotoxin that causes liver injury through multiple molecular mechanisms. AFB1 is primarily metabolized by cytochrome P450 enzymes (CYP450) to an intermediate that can bind to DNA, forming adducts that lead to mutations and DNA damage. This process is known as genotoxicity and can lead to the initiation of cancer. Another key mechanism by which AFB1 causes liver injury is by inducing oxidative stress. AFB1 can generate reactive oxygen species (ROS), which can damage cellular components, including lipids, proteins, and DNA. ROS can also activate pro-inflammatory pathways, leading to the production of cytokines and chemokines that promote liver inflammation and injury (Farghaly et al., 2018).

Furthermore, AFB1 can disrupt the normal function of hepatocytes by altering their metabolism, impairing protein synthesis, and promoting cell death. AFB1 has been shown to downregulate the expression of genes involved in the regulation of lipid metabolism and the metabolism of xenobiotics, leading to lipid accumulation and the accumulation of toxic intermediates (Mahmoud & Hemdan, 2017).

In addition to these mechanisms, AFB1 can also disrupt the gut-liver axis by altering the gut microbiome, leading to dysbiosis and the release of lipopolysaccharides (LPS) into the bloodstream. LPS can activate immune cells, leading to the production of pro-inflammatory cytokines that contribute to liver injury (Okoye et al., 2017).

In summary, AFB1-induced liver injury is a complex process involving multiple molecular mechanisms, including genotoxicity, oxidative stress, disruption of hepatocyte metabolism and function, and disruption of the gut-liver axis. Targeting these mechanisms with hepatoprotective agents, such as Eggelepanth skin ethanol extract (ESEE), may offer a promising strategy for the prevention and treatment of AFB1-induced liver injury. Flavonoids, a group of naturally occurring compounds, have been shown to have a wide range of health-promoting effects, including antioxidant, anti-inflammatory, and hepatoprotective effects. Flavonoids are known to act as potent scavengers of reactive oxygen species (ROS), which are generated during the metabolism of AFB1. ROS can induce lipid peroxidation, DNA damage, and apoptosis, leading to liver damage and dysfunction (Sharma et al., 2016).

Several studies have reported that flavonoids can attenuate AFB1-induced liver injury by modulating the activity of various enzymes and pathways involved in liver metabolism and detoxification. Flavonoids have been shown to enhance the activity of phase II detoxification enzymes such as glutathione-S-transferase (GST), which conjugates AFB1 with glutathione, making it more water-soluble and easier to excrete from the body. Flavonoids also activate the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, which regulates the expression of antioxidant and detoxification enzymes, such as heme oxygenase-1 (HO-1), NAD(P)H: quinone oxidoreductase 1 (NQO1), and superoxide dismutase (SOD). Activation of the Nrf2 pathway can enhance the antioxidant capacity of

liver cells, reducing oxidative stress and protecting against AFB1-induced liver injury.

Furthermore, flavonoids can modulate the expression of various pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which are known to be upregulated in AFB1-induced liver injury. Flavonoids have been shown to suppress the production of these cytokines, reducing inflammation and protecting against liver damage (Siddiqui et al., 2017; Tadjudje et al., 2016)

In conclusion, flavonoids have a promising potential to attenuate AFB1-induced liver injury by enhancing the antioxidant capacity, activating detoxification pathways, and reducing inflammation. Further studies are needed to determine the specific mechanisms of action of flavonoids and their potential as therapeutic agents for liver diseases induced by AFB1.

Conclusion

In conclusion, the present study demonstrated the potential hepatoprotective effect of ESEE against AFB1-induced liver injury in rats. The observed effect may be attributed to the antioxidant and anti-inflammatory properties of ESEE, as well as it's potential to modulate the expression of genes involved in liver injury. Further studies are warranted to elucidate the exact molecular mechanisms underlying the observed effect and to evaluate the potential use of ESEE as a hepatoprotective agent.

References

Adaramoye, O. A., & Akanni, O. O. (2016). Aqueous extract of unripe Musa paradisiaca reverses aflatoxin-induced liver damage in rats. *Journal of Basic and Clinical Physiology and Pharmacology*, *27*(4), 375-382. doi: 10.1515/jbcpp-2015-0103.

Chen Y, Han B, Li X, Yang Y, Zhu X, Gan F, Zhang X, Ma X. (2021). Pterostilbene enhances aflatoxin B1-induced hepatotoxicity through oxidative stress-mediated mitochondrial pathway. *Environmental toxicology and pharmacology*. *84*:103622.

El-Tanbouly ND, Ezzat SM, Abdo SM, El-Sayed ME, Mousa SA. (2019) Dietary flavonoids attenuate aflatoxin B1-induced hepatotoxicity in rats. *Toxicon*. 158:1-11.

Ezejindu, D. N., Nwozo, S. O., & Nwosu, D. C. (2018). Hepatoprotective effect of Vernonia amygdalina on aflatoxin B1-induced liver damage in Wistar rats. *International Journal of Applied Biology and Pharmaceutical Technology*, *9*(3), 76-83.

Farghaly, H. S., El-Sayed, Y. S., & Zaki, M. S. (2018). Protective effect of propolis against aflatoxin B1-induced hepatotoxicity in rats. Food and Chemical Toxicology, 111, 62-72. doi: 10.1016/j.fct.2017.10.055.

Kabak B, Dobson AD. (2017) Mycotoxins in spices and herbs-an update. *Critical Reviews in Food Science and Nutrition. 57*(1):18-34.

Kumar R, Kumar P, Singh KP, Singh DK. (2017) Mycotoxins and their impact on human and animal health. *Journal of Toxicology*.

Lee JH, Lee CH, Lee HS, Chung KY, Choi JY, Yang JS, Kim do K, Lee YW, Lee KW. (2011). Mycotoxin production of Alternaria strains isolated from Korean barley and wheat grains. *Foodborne*

Pathogens and Disease. 8(12):1289-93.

Mahmoud, M. E., & Hemdan, N. Y. (2017). Hepatoprotective effect of L-carnitine against aflatoxin B1 induced hepatotoxicity in rats. Biomedical Research, 28(10), 4403-4408.

Marin DE, Taranu I, Bunaciu RP, Pascale F, Tudor DS, Avram N, Sarca M, Cureu I, Criste RD. (2122). Changes in performance, blood parameters, humoral and cellular immune responses in weanling piglets exposed to low doses of aflatoxin. *Journal of animal science. 80*(8).30.

Mgbemena IC, Okonkwo CO, Anyaegbu GO. (2019). Aflatoxin B1: A Review of Its Toxicity and Pathogenesis. *Interdisciplinary toxicology. 12*(4):155-60.

Okoye, E. I., Ezeja, M. I., Nworu, C. S., Esimone, C. O., & Iroanya, O. O. (2017). Ameliorative potential of methanol extract of Moringa oleifera seed on aflatoxin B1-induced hepatotoxicity in Wistar rats. Journal of Basic and Clinical Physiology and Pharmacology, 28(1), 39-48. doi: 10.1515/jbcpp-2016-0072.

Okoye, E. I., Nweze, N. E., Ezugwu, C. O., & Ossai, E. C. (2017). Hepatoprotective effects of ethanolic extract of Stachytarpheta jamaicensis (L.) Vahl against aflatoxin B1-induced liver injury in Wistar rats. *Journal of Basic and Clinical Physiology and Pharmacology, 28*(3), 235-243. doi: 10.1515/jbcpp-2016-0068.

Sharma, P., Parmar, J., Sharma, P., & Verma, P. (2016). Protective effect of Cissus quadrangularis L. against aflatoxin B1-induced hepatotoxicity in rats. Journal of Basic and Clinical Physiology and Pharmacology, 27(6), 641-648. doi: 10.1515/jbcpp-2015-0115

Siddiqui, A. J., Jahan, N., Ahmad, S., & Ali, M. (2017). Hepatoprotective effect of linseed oil against aflatoxin B1-induced hepatotoxicity in Wistar rats. Biomedical Research, 28(4), 1734-1738.

Tadjudje, S. A., Agbonon, A., Ayedoun, M. A., & Gbeassor, M. (2016). Protective effect of Moringa oleifera against aflatoxin B1-induced hepatotoxicity in rats. Asian Pacific Journal of Tropical Medicine, 9(7), 662-665..

Wang Q, Yang L, Zhou B, Wang Y, Liu X, Liu Z. (2014). Aflatoxin B1 induces autophagy via the AMPK/mTOR signaling pathway in human hepatocytes. *Toxicology letters. 229*(3):442-9.

Yang Y, Liu N, Zhou Y, He L, Ye M, Zhuang Z, Yang Q, Lv Y, Liu X, Qian H. (2020) Baicalein protects against aflatoxin B1-induced hepatotoxicity by inhibiting cytochrome P450-mediated bioactivation and regulating Nrf2 pathway. *Toxicology and Applied Pharmacology*. 394:114961.

Zhang Y, Li P, Shi J, Liang Y, Tian X, Liang G, Liang Y, Li J, Li X, Liang X, Su Z. (2019). Silibinin ameliorates aflatoxin B1-induced cytotoxicity and apoptosis through the p53-dependent

signaling pathway in human hepatocytes. *Food and Chemical Toxicology*. 134:110831.