Characterization of Aflatoxin B1 Biomarkers as Preference for Antitoxin Candidates for Biosensors for Halal Food Supply

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Abstract

The fungus grows on various types of food and feed, its growth will cause damage, including damage to flavor, color, softening, and the formation of toxic compounds. The damage is because the fungus produces extracellular enzymes that break down certain compounds in food and feed, and produce toxic secondary metabolites, called mycotoxins. Fungal contamination requires serious attention, not only because it causes damage to food and feed but is related to the potential of the fungus to produce mycotoxins and form conidia that are pathogenic or allergic, mycotoxins produced by various types of fungi, each of which has varying toxicity, in general chronic causes mycotoxicity. Mycotoxins have caused several types of diseases in humans and animals. Aflatoxin B1 (AFB1) is a potential toxin as a hepatocarcinogen. Eating foods tainted with AFB1 can cause acute (short term) and chronic (moderate or long term) poisoning; chronic disorders such as disorders of the central nervous system, cardiovascular and lung systems, and digestive tract. Some mycotoxins are carcinogenic, estrogenic, mutagenic, teratogenic, and immunosuppressive. The purpose of this study is the characterization of Aflatoxin B1 Biomarkers as Preference for Antitoxin Candidates for Biosensors for Halal Food Supply. The method of this research is AFB1 induction in mice, observed for 14 days, on the 14th day liver organs were taken. The liver is prepared into organ extracts, analyzed using IHC. From the research results obtained protein expression in the liver organs of experimental animals by the IHC method. Further research is needed as an antitoxin and biosensor.

Keyword: *Aflatoxin B1, MAPK1, AKT1, Antitoxin.*

Introduction

Pathophysiologically Aflatoxin B1 (AFB1) as a carcinogenic material arises because of chronic exposure to hepatocyte cells that cause epigenetic and genetic changes that lead to the induction of oncogenic proteins and/or activation of tumor suppressor genes. This pathogenesis occurs in four important signaling pathways (WNT, TGFß, PI3K / Akt, and RAF / MEK / ERK)[1].

The RAS / RAF / MEK / ERK pathway plays an important role in the development of liver cancer. Similar to many signaling networks, this pathway is activated through a variety of mechanisms. RAS activation is initiated by the binding of extracellular signaling compounds such as hormones and growth factors with VEGFR and PDGFR. The bond between signaling compounds will cause a phosphorylation reaction in the RAS and initiate a series of cascades that lead to the activation of ERK for proliferation and differentiation and angiogenesis. Certain mutated components or Ras-Raf-MEK-ERK / MAPK overexpression are increasingly being studied in carcinogenesis. The abnormal target protein signaling pathway contributes to cell proliferation, differentiation, survival, and uncontrolled cell apoptosis is a biomarker of carcinogenic processes [2].

The PI3K pathway is activated through various mechanisms such as the binding of IGF-1 to IGFR so that it starts the P13K signaling series and activates AKT which directly influences mTOR which will regulate proliferation and angiogenesis[1].

Hepatocellular carcinoma (HCC) is a malignant

tumor that attacks the liver. The prevalence of malignant tumors is very high, ranked fourth globally as cancer with an incidence rate of 5.3% compared to other cancers[3]. Barriers from HCC signaling compounds such as MAPK1 (ERK) and AKT using special interventions can inhibit the proliferation and angiogenesis of HCC and reduce the concentration of these compounds due to excessive expression due to genetic and epigenetic factors[4].

Based on the results of in silico research, there is a super-expression of AKT-1 and MAPK-1 evidence as a molecular mechanism pathway for AFB-1 carcinogenic material in hepatocyte cells. Analysis of amino acid residues, against AKT-1 and MAPK-1, have residues at the Binding site that affect receptors on oncogenic proteins[5].

From this background, this study aims to test the exposure of AFB1 to the expression of AKT1 and MAPK1 target proteins using the Immunohistochemistry (IHC) method to determine the reaction between receptor-ligand (antigen-antibody) complexes formed in vivo tests.

Material and Methods

This study used an experimental laboratory research design with experimental animals (In vivo) with an examination of the immunohistochemical method.

Results and Discussion

Immunohistochemistry (IHC).

Immunohistochemical examination is intended to determine the expression of AKT 1 and MAPK1 (ERK) on cells of mice hepatocytes (Mus muscullus). AKT 1 and MAPK 1 (ERK) expression score data were obtained using the modified Remmele method[6], Remmele scale index (Immuno Reactive Score / IRS) is the result of multiplying the percentage score of immunoreactive cells with the color intensity score on immunoreactive cells. Data for each sample is the average value of the IRS observed in 5 (five) Field View (LP) at 400x magnification.

Comparison of AKT 1 expression (chromogen brown) in hepatocyte (arrow) cells between treatment groups. AKT1 is expressed in both the cytoplasm and the nucleus of hepatocyte cells.

The results of the examination showed that the expression of AKT1 in the treatment group giving AFB1 (3 mg / Kg BW) (P1) seemed stronger than the control treatment group (P0).

Comparison of MAPK1 (brown chromogen) expression in hepatocyte (arrow) cells between treatment groups. MAPK1 is expressed in both the cytoplasm and the hepatocyte cell nucleus. The results of the examination showed that the expression of MAPK1 in the treatment group Giving AFB1 (3 mg / Kg BW) (P1) seemed stronger than the control treatment group (P0).

The data obtained in the form of a Remmele Scale Index score (Immuno Reactive Score / IRS) results from the multiplication score of immunoreactive cells with a color intensity score, were analyzed by Kruskal Wallis followed by the Z Test, statistical analysis using the IBM SPSS Corp. computer statistical program. Real 21.

Statistical analysis, data are presented as mean (mean) ± standard error. Differences between groups were assessed for statistical significance using the Kruskal-Wallis test or the Multiple comparison test with the Z test (Multiple comparisons by Z test), depending on data distribution. P values <0.05 were considered statistically significant.

The data obtained indicate the number of assessment scores with the Immuno Reactive Score (IRS) on the expression of AKT 1 under each treatment condition (mean ± SEM of six replications). Statistically significant difference from controls (* $p<0.05$; ** $p<0.01$).

The data obtained shows the number of assessment scores with Immuno Reactive Score (IRS) on the MAPK 1 expression under each treatment condition (mean ± SEM of six replications). Statistically significant difference from controls (* $p<0.05$; ** $p<0.01$).

The highest MAPK1 expression in AFB1 (P1) treatment, with a mean rank of 18.41 ± 1.4108 showed a significant difference ($p<0.05$) with a control treatment (P0) with a mean rank of 8.75 ± 3.5137. It was also found that the treatment was significantly different ($p<0.05$).

Based on the results of testing using Kruskal Wallis can provide information that the administration of

AFB1 can increase the expression of AKT 1 and MAPK 1 on mouse hepatocytes induced by AFB1 in super expressions Vivo administration so that it can be used as individual biomarkers exposed to AFB1.

The immunohistochemistry technique is a method that aims to identify specific cells based on antigenic components or cellular products with complex antigen-antibody reactions. Immunohistochemistry is used as a basis for diagnosis and identification of cell types based on cytomorphology. This examination is often done in cases of tumors or malignancies[7,8]. Also, immunohistochemistry is often used for research to determine the distribution and location of biomarkers or expressed proteins in various body tissues[9].

Immunohistochemistry is a combination of histological or cytological examination with immunology. The method of coloring substances or active ingredients in tissues uses the basic principle of immunology, namely by binding the active ingredients or antigens on the specific active side by the active ingredients or antibodies. The results of this reaction can be identified in the specimen if the antibodies are bound by a marker that can be in the form of fluorescein, enzymes, particulate matter, or isotopes that can be visualized, to indicate the presence of active ingredients in the tissue. Active ingredients can be in the form of proteins, carbohydrates, nucleic acids, fats, other natural ingredients and synthetic materials[10,11].

The immunohistochemical examination has a high ability to separate, select, and be specific. This examination is to detect the presence of antigens, this is due to the presence of specific bonds between antigens and antibodies [12,13,14.]

The results of this study obtained a reaction to AKT1 and MAPK-1 based on immunohistochemistry. Also, based on the results of Scoring with Immuno Reactive Score / IRS showed the expression of MAPK 1 in each treatment.

This shows that if AFB1 enters the body it is predicted to potentially influence the activation of the AKT1 and MAPK1 oncogenic protein activation, so that it triggers the regulation of the underlying process (down-regulation) so that it can trigger cancer cells in the hepatocellular carcinoma (HCC). Capsaicinoids include nordihydrocapsaicin, capsaicin, dihydrocapsaicin, nor capsaicin, homodihydrocapsaicin, homocapsaicin, nonivamide[15]. One of the most important compounds in chili is capsaicin, capsaicin is a secondary metabolite of the chili plant. In the pharmaceutical field in addition to relieving pain or pain, capsaicin is also known to have anticancer activity because it inhibits certain oncogenic proteins[16]. AFB1 has binding energy that is more stable compared to other proteins so it allows the formation of the AFB1-AKT1 molecule complex. It is predicted that AKT1 is one of the oncoproteins so that there is an AFB1 barrier indirectly interfering with activation of the AKT1 and MAPK1 proteins.

Based on the results of in silico research, super-expression of AKT-1 and MAPK-1 occurs in AFB1-induced cells, this shows evidence as a molecular pathway mechanism and can be used as a marker as making antitoxin (anti-AFB1) against AFB-1 as an effort in searching biosensors in the context of halal and thoyib food products.

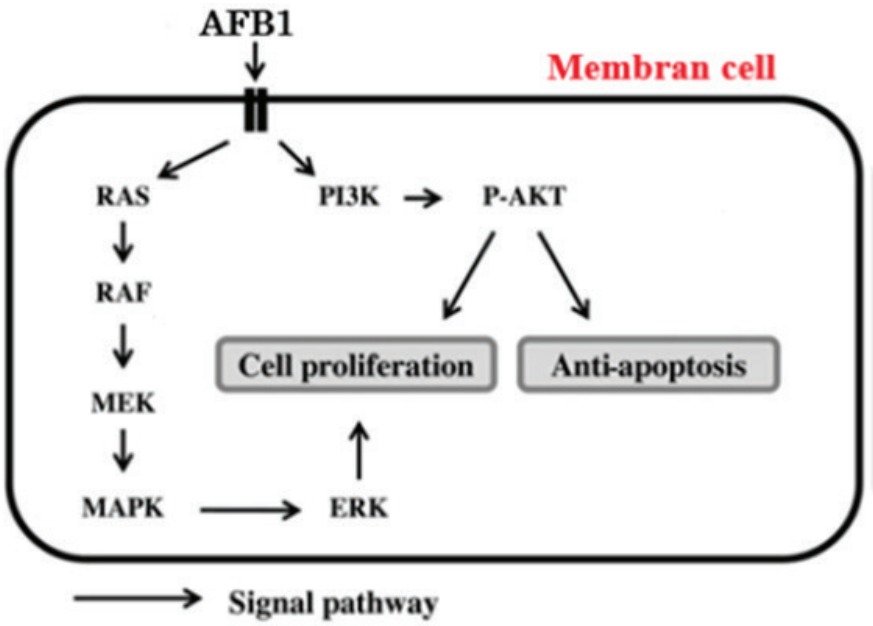


Figure 1. Schematic diagram of the cellular mechanism AFB1 in the RAS / RAF / MAPK pathway and the PI3K-AKT pathway that occurs super expression[17] (*modification*).

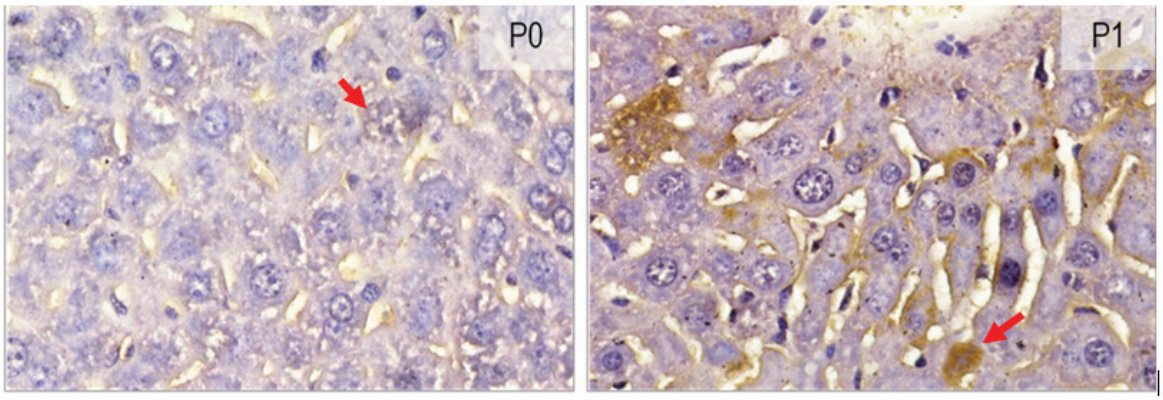


Figure 2. Immunohistochemical staining, 1000x magnification; microscope Nikon H600L; DS Fi2 300 megapixel camera.

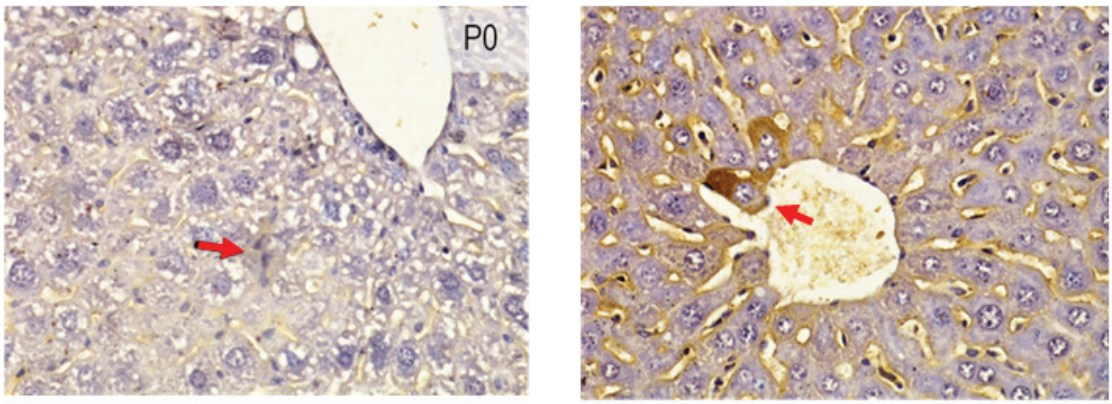
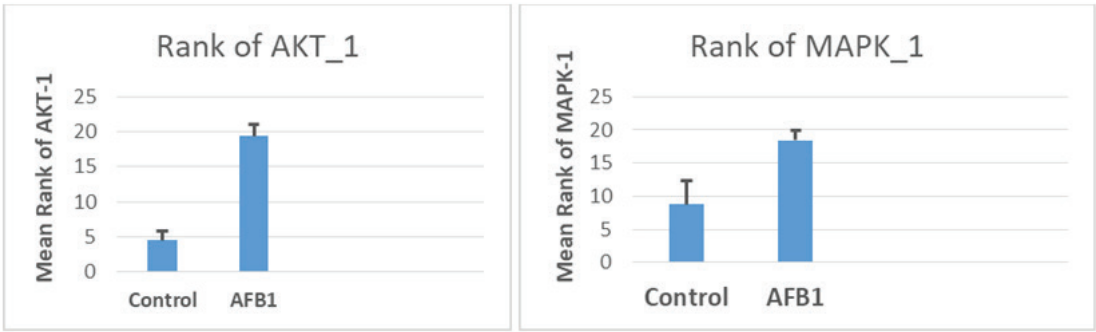


Figure 3. Immunohistochemical staining, 1000x magnification; Nikon H600L microscope; DS Fi2 300 megapixel camera.

Table 1. AKT-1 and MAPK-1 Rank Table

				AKT_1	MAPK_1	Rank of AKT_1	Rank of MAPK_1
P	Kontrol	1		2.2	4.6	2.0	6.0
		2		1.6	4.4	1.0	4.5
		3		4.0	2.6	10.0	2.0
		4		2.6	2.4	4.0	1.0
		5		2.4	8.0	3.0	21.5
		6		3.6	6.8	7.0	17.5
			Mean	2.733	4.800	4.500	8.750
		Total	Std. Error of Mean	.3676	.9136	1.3844	3.5137
	AFB1	1		6.4	6.4	20.0	15.5
		2		7.8	6.2	23.5	14.0
		3		6.0	8.2	19.0	23.0
		4		5.8	6.8	18.0	17.5
		5		4.2	7.0	12.0	19.0
		6		7.8	8.0	23.5	21.5
			Mean	6.333	7.100	19.333	18.417
		Total	Std. Error of Mean	.5554	.3376	1.7401	1.4108



A B

Figure 4. Average data diagram (mean) ± standard error AKT 1 (A) and average data (mean) ± standard error MAPK 1 (B) with group differences for significance statistics.

Conclusion

In conclusion, our data show that AFB1 can synergistically react through the anti-apoptotic AKT signaling pathway and MAPK 1 to potentiate antiproliferative actions that can promote apoptosis, so that it is suspected to have potential as a marker in the manufacture of antitoxin candidates in the search for biosensors in the framework of biosensor food products Halal and Thoyib, a finding that demands further clinical testing.

Suggestion: Conducting further tests and standardization of formulations in the manufacture of antitoxin as a biosensor against AFB1.

Ethics and Consent: All applicable institutional guidelines for the care and use of animals were followed. This research received ethical clearance approval from Animal Care and Use Commitee, Faculty of Veterinary Medicine, Universitas Airlangga Surabaya, East Java, Indonesia (No. 1.KE..198. 12. 2019).

Confilct of Interests: The authors declare that they have no conflict interests

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