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The Ameliorative Effect of Apigenin in *Plectranthus amboinicus* Lour Spreng in the Treatment of Hepato-renal Carcinogenesis Induced Benzo(a)pyrene

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Abstract

Benzo(a)pyrene (B(a)P) is the major cause of hepato-renal carcinogenesis. Apigenin in *Plectranthus amboinicus* (EPA), has indicated some biological activities such as antioxidant and antimutagenic activity. The aim of this study is to investigate the potential of apigenin in EPA as anti-cancer against chronic hepatorenal damage exposed to B(a)P. The rats of 4 groups (n=6) were divided as follows: Group I (P0) was given food and water ad-libitum; Group II (PB) was administered orally B(a)P 2 mg/kg BW; Group III (PB+E) received orally B(a)P 2 mg/kg BW and EPA 500 mg/kg; Group IV (PE) was administered orally EPA 500 mg/kg BW. The therapeutic effect of EPA was explored using network pharmacology and molecular docking. The results showed that Group III could significantly improve ($P < 0.05$) the hepatorenal function parameter, including DNA concentration. SGPT, SGOT, blood urea nitrogen, and creatinine compared to those treated with B(a)P. The outcome data pharmacology revealed 6 targets could be the main core target. The good binding affinity indicated Apigenin docked to AKT1 protein with -10.00 kcal/mol relevant to Doxorubicin as control drug. Our results provide a new insight of apigenin in EPA potentially suppressing the regulation of chemical carcinogenesis by B(a)P.

Keywords: Apigenin; Benzo(a)pyrene Liver Kidney carcinogenesis.

INTRODUCTION

Benzo(a)pyrene (B[a]P) is a class I carcinogen found in tobacco, contaminated water, and high temperature processed food (Arifin et al., 2022). The xenobiotic compound from Polycyclic aromatic hydrocarbons (PAH) potentially tends to be a marker for genotoxic and carcinogenic development (Saputro et al., 2021). Liver and kidney are the crucial target responded to B(a)P toxicant. Metabolic homeostasis interfered by B(a)P exposure could generate the ferroptosis in cell death and kidney dysfunction (H. Chen et al., 2025; Gong et al., 2025; Hao et al., 2024; Shaoyong et al., 2023; Zhang et al., 2025). The B(a)P oxidation is catalyzed by cytochrome P45 to result dihydrodiol, epoxides, and dihydrodiol epoxides in liver (Ahamed et al., 2023; Zhao et al., 2024). It bound covalently to DNA, for example N- amino guanine and a phosphate group from nucleic acid (Feng et al., 2022). Besides, this alteration of DNA structure accumulated the production of 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-benzo[a]pyrene (BPDE) as an important key for the initiate cell mutagenesis (Gerhards et al., 2023). The previous research suggested that the BPDE-DNA adducts promote the production of oxidative stress in human renal cells (J. Chen et al., 2023). DNA methyltransferase 1 (DNMT1) is inhibited by B[a]P that resulted the DNA hypermethylation in tumor development (Hoff et al., 2023; D. Sun et al., 2022; Wang et al., 2024).

Despite several treatments for hepato-renal carcinogenesis have been investigated, the percentage of mortality is higher (Sinaga et al., 2023). The pharmacological modern analysis, such as network pharmacology and molecular docking, had been employed previously to understand the biological activities of active compounds against numerous diseases. The research of phytocompounds effectively could determine the molecular mechanism pathway as a promising new treatment (Jadhav et al., 2025; Liang et al., 2024).

In recent years, chemotherapeutic natural compound tends to be a promiscuous treatment of hepato-renal carcinogenesis (Scaria et al., 2020). *Plectranthus amboinicus* is an edible plant from family Lamiaceae (Ślusarczyk et al., 2021). The herbal medication was

applied for various treatment purpose such as cough, nasal congestion, bronchitis, hepatopathy, and rheumatism (Stasińska-Jakubas et al., 2023). Apigenin is a dietary flavonoid compound found abundantly approximately 2.3 ng/µl in P. amboinicus (Silitonga et al., 2015). The pharmacological properties of apigenin have anti-apoptotic (Mahbub et al., 2022), pro-oxidation properties (Warkad et al., 2021), inhibitor tumour proliferation, anti-genotoxic, hepato-renal protective (Singh et al., 2022), antioxidant, and anti-inflammatory (Kashyap et al., 2018). However, there is limited data about the role and mechanism of apigenin in EPA to treat hepatorenal carcinogenesis.

The aim of this study was to validate the potential of Apigenin in *Plectranthus amboinicus* by identifying DNA concentration, hepatorenal functional and discovering system pharmacology and molecular interaction for the development of cancer treatment. We suggest DNA fragmentation and hepato-renal functional parameter to estimate the significant factors damaged by toxic chemical in rats induced B(a)P (Akintunde et al., 2020; Sugawara et al., 2022). Additionally, our studies also predict the potential apigenin against hepatorenal cancer by pharmacological modern approaches, such as network pharmacology and molecular docking study. This method is a very effective methodology to demonstrate the pathway of cancer treatment with less toxicity and adverse side effect (J.-J. Chen et al., 2021).

MATERIALS AND METHODS

Preparation of Plant Material.

Plectranthus amboinicus were obtained from North Tapanuli, North Sumatra- Indonesia (Specimen number: UNIMED082022). The sample was air-dried for 7 days, powdered into 1000 g, and extracted with 96 % ethanol. The extraction was soaked for 5 days and stirred two times a day. The extracts were filtered by Whatman filter paper and concentrated by using a rotary evaporator, following the previous maceration method (Silitonga et al., 2018).

Animal and Experimental Design

The scientific procedures were approved by the State University of Medan Ethics Committee (ID: 0453/EPH-FMIPA/2019). The current experiments were based on the World Medical Association (WMA) statement on Animal Use in Biomedical Research and EU Directive 2010/63/EU. Twenty- four male Wistar rats (aged 8 weeks) were obtained from Pharmacy Laboratories USU, Indonesia. Animals were acclimatized in a temperature – controlled animal (22±20C) for seven days and were given free access to feed and water ad-libitum at Biology Laboratories UNIMED, Indonesia. The experimental animals were treated for twenty- two days and divided to four groups (six rats per group); Group I (P0) received food and water ad-libitum as a control group; Group II (PB) administered orally B(a)P 2 mg/kg BW; Group III (PBE) received orally B(a)P 2 mg/kg BW and EPA 500 mg/kg; Group IV (PE) administered orally EPA 500 mg/kg BW. At the end of the experimental time (23 days), all the group animals were euthanized. The blood sample was taken from cardiac puncture and put into the Eppendorf tube with EDTA anticoagulant. Serum was separated by centrifugation at 3000 rpm for 5 minutes. The liver and kidneys were washed in ice-cold normal saline solution and kept for further DNA concentration.

DNA Fragmentation assay

The cell of the sample was isolated from blood, liver, and kidney tissues. The procedure was adapted by following Manual Instruction Kit Geneaid gSYNCTM Ver. 02.10.17, consist of tissue dissociation, cell lysis, DNA binding, washed and elution. Then, the DNA was isolated from the cell lysates and quantified by using UV Spectrophotometric measurement at 260 nm (Soumya et al., 2021).

Hepatorenal function analysis

Hepatorenal function parameters consist of SGPT (Serum Glutamic Pyruvate Transaminase), SGOT (Serum Glutamate Oxalate Transaminase), creatinine, and blood uremic nitrogen value (Bokhary et al., 2022). Serum solution (100 µl) was put into commercially reagent kits 1000µl and incubated at 370 C for 5 minutes (Aljarba et al., 2021). SGPT and SGOT were quantified by a photometric method at 340 nm. Total protein assay is measured using Creatinine kit test (CREA) and Blood Urea Nitrogen kit (BUN) (Gwon et al., 2021).

Statistical analysis

The average data are presented as the mean ± SD. The significance between B(a)P treated and EPA treated groups were evaluated by the Analysis of Variance (ANOVA) method. The value $P < 0.05$ was considered as statistically significant.

Bioinformatic studies

The String v_11.0 database and Cytoscape ver. 3.9.1 were used to develop a network between apigenin and hepato-renal cancer genes. The settings for the network were established and adapted with the organism "*Homo sapiens*" and the confidence was set at 0.7 (Sinaga et al., 2024). The nodes represented proteins and the edges indicated the interaction protein. Furthermore, the node's colour was evaluated by the parameter betweenness centrality to identify the genes which contributed in the cancer pathway using the Cytoscape data. The biological pathway was evaluated by STRING 11.5 database (L. Sun et al., 2023).

The 3D structure of apigenin and doxorubicin as control drugs in "sdf" format were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Protein AKT (PDB: 2UZW) was downloaded from RCSB

(https://www.rcsb.org/). All apigenin, doxorubicin, and protein were prepared to remove waters, ions, and ligands, then optimized by the MMFF94D force field. All of them were saved in "pdbqt" format. Molecular docking study was conducted with AutoDock Vina software (Khalil et al., 2022). The visualization was displayed by using BIOVIA Discovery studio software.

RESULTS AND DISCUSSION

Results

B(a)P induces DNA damage in hepato-renal cells.

To evaluate the potential of ethanol extract of *Plectranthus amboinicus*, we assessed DNA concentration in hepato-renal damage induced B[a]P. The high levels of DNA concentration in renal tissue have a significant ($p<0.05$) augmented in renal tissues of the B(a)P treated group compared to all the treatment groups (**Fig. 1a**). After the administration of EPA, DNA concentration could be normalized to the relevant with the control group. The following result demonstrate the DNA concentration value reduction ($p<0.05$) was shown in the administration EPA groups compared to the B(a)P treated group in liver tissues (**Fig. 1b**). Occurrence of DNA damage was detected by UV spectrophotometer at 260 nm as biochemical signal of apoptosis in hepato-renal cells at **Fig. 1c, d.**

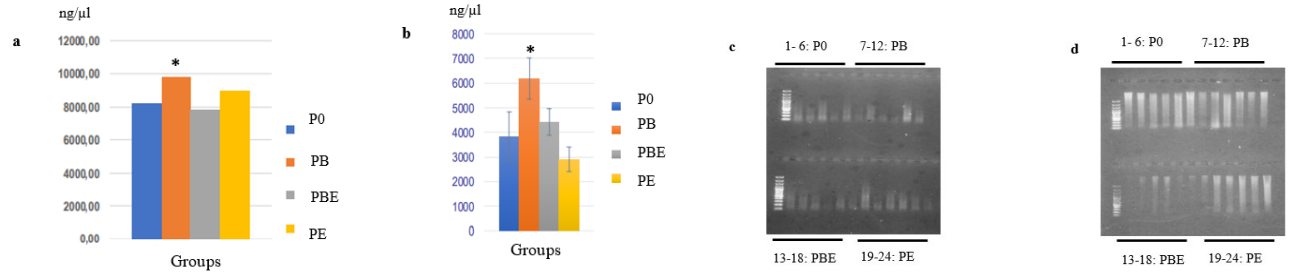


Figure 1. The effect of apigenin in *P. amboinicus* against hepato-renal carcinogenesis induced B(a)P; (a) DNA Concentration in renal; (b) DNA Concentration in liver; (c) DNA fragmentation in renal; (d) DNA fragmentation in liver; (P0) the control group; (PB) B(a)P 2 mg/kg BW; (PBE) B(a)P 2 mg/kg BW + EPA 500 mg/kg; (PE) EPA 500 mg/kg BW. (n=6, $^{*}p < 0.05$).

Evaluation of hepatorenal parameter in rats induced B(a)P.

The average of SGOT and SGPT levels in the blood serum of all groups is depicted in **s**. Our finding indicated that B(a)P significantly increased SGPT and SGOT levels as compared to the control group (P0). However, the administration of EPA significantly reduced ($p<0.05$) in comparison to the B(a)P treated group. We hypothesized that creatinine and uremic were significantly higher after B(a)P administration throughout the experimental period of 23 days. However, the ethanolic extract of *Plectranthus amboinicus* significantly alleviated ($p<0.05$) the creatinine and uremic levels in the PBE group as compared to the B(a)P treated group (**Fig. 2c, d**).

Bioinformatic studies.

The network pharmacology with confidence scores 0.4 was built. The 43 nodes and 389 edges were obtained and 6 intersecting targets of apigenin, for instance TP53, AKT1, MTOR, CCND1, GGT1, and ESR1, were identified as the main protein which play an important role in the cancer pathway (**Fig. 3**). The biological processes of apigenin were shown in **Table 1**. We observed the target involved in oncogenesis, consist of P53 signaling pathway, PI3K-AKT signaling pathway, positive regulation of chemical carcinogenesis by Benzo(a)pyrene, TNF signaling pathway, HIF-1 signaling pathway, and FoxO signaling pathway.

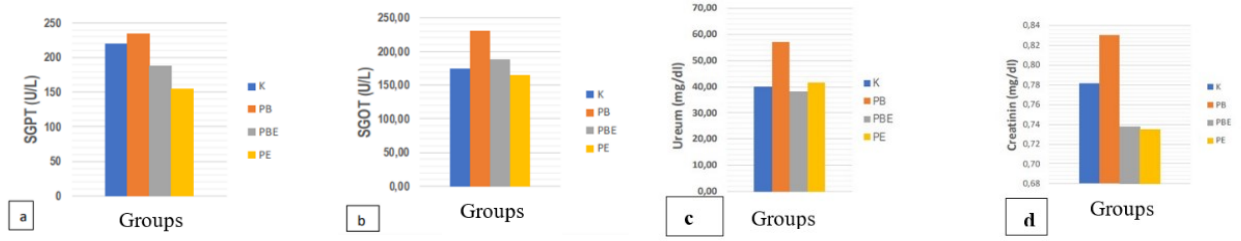


Figure 2. The therapeutic potential of apigenin in *P. amboinicus* against hepato-renal carcinogenesis induced B(a)P (a) SGPT and (b) SGOT levels; (c) Ureum; (d) Creatinine (P0/K) the control group; (PB) B(a)P 2 mg/kg BW; (PBE) B(a)P 2 mg/kg BW + EPA 500 mg/kg; (PE) EPA 500 mg/kg BW. (n=6, $^{*}p < 0.05$).

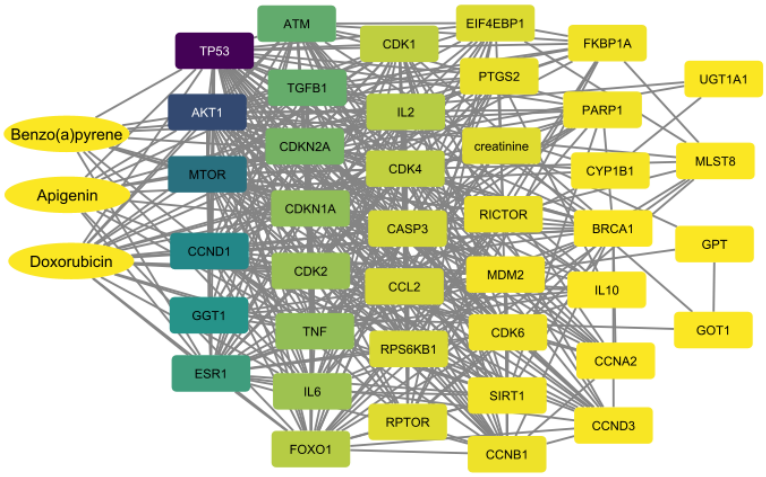
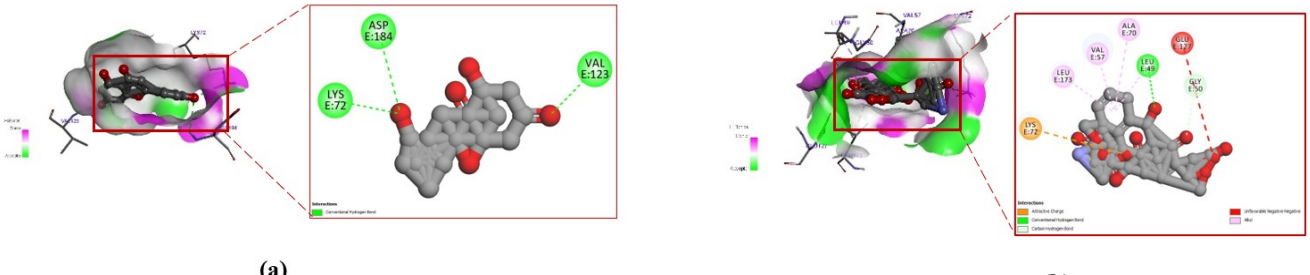


Figure 3. The network pharmacology construction of Apigenin in *P. amboinicus* against hepato-renal carcinogenesis induced B[a]P. The higher degree value is displayed by color ranging from dark to light.

Table 1. Biological Pathway of Apigenin in *Plenctranthus amboinicus* againts hepato-renal carcinogenesis

Pathway ID	Pathway description	Count in gene set	Gene	False discovery rate
5206	MicroRNAs in cancer	14	CYP1B1, MDM2, CASP3, BRCA1, CDK6, ATM, CDKN1A, MTOR, CDKN2A, PTGS2, TP53, SIRT1, RPTOR, CCND1	6.56e-18
4115	P53 signaling pathway	11	MDM2, CDK6, CDK4, CCNB1, ATM, CDKN1A, CASP3, CDKN2A, TP53, CCND3, CCND1	1.25e-16
5200	Pathway in cancer	14	MDM2, CASP3, FOXO1, CDKN1A, CDK6, CDK4, AKT1, MTOR, CDKN2A, TGFB1, PTGS2, TP53, CCND1, MDM2	9.86e-14
4068	FoxO signaling pathway	11	MDM2, CCNB1, FOXO1, ATM, CDKN1A, AKT1, TGFB1, IL10, IL6, SIRT1, CCND1	4.99e-14
4151	PI3K-Akt signaling pathway	16	TP53, MTOR, CDK4, AKT1, CDKN1A, CDK6, BRCA1, MDM2, IL2, IL6, CCDN3, CCND1, RPTOR, EIF4EBP1, MLST8, RPS6KB1	3.34e-16
4210	Apoptosis	5	TP53, AKT1, TNF, CASP3, ATM	7.66e-06
4668	TNF signaling pathway	6	TNF, PTGS2, CCL2, AKT1, CASP3, IL6	9.66e-07
5204	Chemical carcinogenesis	3	PTGS2, CYP1B1, UGT1A1	0.00214
4066	HIF-1 signaling pathway	6	CDKN1A, AKT1, MTOR, EIF4EBP1, RPS6KB1, IL6	8.46e-07

The interaction between apigenin and the promising target were evaluated by molecular docking analysis. Apigenin could be a promising compound which bound to AKT1 with good binding affinity at -10.00 kcal/mol as well as control drug anti-cancer that is doxorubicin at -12.20 kcal/mol in **Table 2**. Here, all the major binding results hydrophobic interaction and hydrogen bond at residue Val57, Lys72, Leu 173 represented in **Fig. 4**.



(a) **(b)**

Figure 4. Molecular docking between (a) Apigenin; (b) Doxorubicin with Protein AKT1.

Table 2. Binding affinity between the compounds and AKT1.

Compound	Binding affinity (kcal/mol)	Hydrophobic interaction	Hydrogen bond
Apigenin	-10.00	Leu49, Val57, Ala70, Leu173, Thr183	Ly72, Val123, Asp184
Doxorubicin	-12.20	Val57, Leu173	Thr51, Lys72, Asp184

Discussion

The identification of ameliorative effect from ethnomedicinal plants is needed to encourage anticancer treatment for hepato-renal carcinogenesis with less side-effect (Silitonga et al., 2024). However, some chemotherapeutic and environmental agents elevated hepato-renal enzyme activity, DNA fragments destruction and abnormalities cell in the hepato-renal cells (Meena et al., 2021). The high index of liver damage, for instance SGPT and SGOT, are caused by the reactive oxidative stress and free radicals (Ablat et al., 2023). Furthermore, the role of renal is to maintain plasma urine and creatinine clearance (Oto et al., 2020), as an important indicator of renal function. In normal renal cells, the markers are resulted from creatine phosphate in the muscles and removed from the urine (Bredahl et al., 2021). Hepato-renal carcinogenesis is enhanced by B(a)P exposure (Lindsay Reed et al., 2020; Wei-Sheng Lin et al., 2023). The therapeutic effect of EPA against hepato-renal damage induced B(a)P is still unclear. Therefore, our observation demonstrated that EPA reduced hepatic and renal dysfunction parameters, consisting of SGPT, SGOT, creatinine and urea, after 23 days exposure to B(a)P. This experimental data agrees to the previous data that apigenin could be a potential compound to prevent hepatic and renal damage in rats exposed to xenobiotic compound (Owumi et al., 2022). DNA damage exposure to B(a)P stimulated systematic response by upregulated signaling pathways in hepatorenal cells. Our finding is similar to the previous research that the accumulation of B(a)P could damage DNA in hepatorenal carcinogenesis (Yining Xiong et al., 2021; Yunxia Han et al., 2023). Within the limits of variability of our data, the DNA improvement appeared more obvious in the PBE group. Additionally, B(a)P induction results in DNA damage trigger accumulation of p53 and release caspase-3 cleavage. This is in line with previous result that mitochondrial apoptotic cell death triggered by p53 activation in phenylpropene methyleugenol (ME)-exposure DNA adducts (Carlsson et al., 2022). The accumulation of B(a)P destructed DNA fragment, particularly hepato -renal, by decreasing gene expression (Garaycoechea et al., 2023; Holmes & Winn, 2022).

We predict a comprehensive approach integrated protein-protein interaction and docking study to investigate mechanism apigenin against B(a)P caused hepatorenal cancer. We discovered that Benzo(a)pyrene tends to play a positive role in chemical carcinogen signaling (Vermillion Maier et al., 2023), which is involved in oxidative stress (Song & Choi, 2021), inflammation (Wu et al., 2022), and apoptosis pathway (Barangi et al., 2020). This result is similar to the previous observation which investigated mechanism of the active compound against B(a)P induced hepatorenal injury (Ge et al., 2022). The main target of apigenin and B(a)P interaction were discovered, including TP53, AKT1, MTOR, CCND1, GGT1, and ESR1. All the targets are significantly relevant in the progression and metastatic hepato-renal cancer. AKT1 is an important protein target associated in apoptosis cells (G. Sun et al., 2020), TNF signaling pathway (Lee et al., 2022), and carcinogen pathway (Yassin et al., 2022). Apigenin has been contributed in modulating the expression of AKT proteins. Apigenin exhibited the protective effects to AkT phosphorylation through disruption of the ATP binding domain in PI3K and activation of mTORC2 complex (Javed et al., 2021). Additionally, Apigenin has a protective property against cancer in rat model through PI3K/Akt/mTOR signaling pathway (Yang et al., 2018). Our finding suggests a new insight into the anti-cancer activity of apigenin in hepatorenal cancer.

CONCLUSION

In conclusion, our results verify that the potential of EPA tends to be anti- cancer activity by preventing hepato-renal damage in B(a)P exposure. The outcome result indicated that SGPT and SGOT significantly reduced ($p<0.05$) in EPA Groups compared to the B(a)P treated group. The creatinine and uremic levels significantly restored in the PBE group after B(a)P exposure. Furthermore, DNA concentration reduction ($p<0.05$) was shown in the administration EPA groups compared to the B(a)P treated group in liver and renal tissues. Our work also reports apigenin of EPA could potentially suppress hepato-renal cancer activity to the protein target, such as TP53, AKT1, MTOR, CCND1, GGT1, and ESR1. Further investigation of the present study, the combination treatments of EPA with doxorubicin at a dose of 0.5 mg/kg are needed to develop a promising therapeutic for cancer.

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Authors' Contributions: Melva Silitonga wrote the original draft and supervised the research. Hendro Pranoto validated the data. Erlintan Sinaga investigated the resources. Feimmy Ruth Pratiwi Sipahutar visualized the bioinformatic data. Adriana Yulinda Dumaria

LumbanGaol designed methodology. Fajar Apollo Sinaga validated the software. All authors read and approved the final version of the manuscript

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