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Original Research Article

The Therapeutic Effect of *Bidens Pilosa* as Anti-Colon Cancer in Alcohol- Induced Rats: *In Vivo* and *In Silico* Method

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ABSTRACT

Bidens pilosa leaves ethanol extract (EBP) has been evaluated for its anti-inflammatory, anti-mycobacterium, and immunomodulatory properties. However, the potential of EBP against colon injury in alcoholic rats remains elusive. In this regard, the potential EBP was investigated for its anti-cancer effect in alcohol-induced colon injury. The EBP with three different doses was treated for six weeks in the alcohol animals. Blood level and colon tissues were examined in alcohol-induced colon. The modern pharmacological analysis was conducted to prove the potential of EBP against colon carcinogenesis. The results defined that alcohol administration significantly disrupted the goblet and crypt cells in the colon tissue. Alcohol decreased the blood parameters; notably, an MCV level of 52.68 ± 1.35 is lower than the control group at 55.5 ± 4.43 ($P < 0.05$). In the alcohol model, EBP supplementation (500 and 750 mg/kg) improved the colon histological damage and restored the blood parameters. The bioinformatic studies exhibited that the EBP bioactive compounds regulated the signaling pathway of main targets, notably CASP3, EGFR, CCL2, and STAT3, in the alcohol-induced colon carcinogenesis. These results are considered to be the underlying reference to investigate the potential EBP against alcohol-induced colorectal injury.

***Keywords*:** Bidens, Alcohol, Colon, Apoptosis, Proliferation, Bioinformatics

Introduction

The risk of colon cancer, including 45% for colon and 49% for rectal carcinogenesis, is linked to heavy alcohol drinking (≥ 25-45 g/day). [1] Alcohol metabolism deactivates tumor-suppressor signals caused by DNA mutations and oxidative stress in the progression of colon cancer. [2] Recent evidence has reported that ADH (alcohol dehydrogenase) enzymes play an important role in increasing colon cancer risk, particularly ADH1B rs4147536 and ADH1C rs283415.[3] This enzyme results in endotoxin metabolites altering the blood alcohol levels and accumulating reactive oxygen species (ROS) in chronic alcohol intake. [4,5] Some studies declare that higher levels of ROS are linked to proliferation and apoptosis. [6–8] The ROS production strongly leads to cancer cell death, which acts as the potential prognostic-associated genes in colon cancer. [9] The inhibition of Wnt/β-catenin, expression of COX-2, and activation of EGFR could be an important key as pro-apoptotic and anti-proliferative effects on numerous tumor-derived cell lines.[10,11]Drug resistance and cytotoxic pharmaceuticals are the challenges to developing the treatment of colon carcinogenesis.[12,13] The effectiveness of CRC treatment with potential targets is still established.

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However, bioinformatic approaches[14], consisting of modern pharmacological analysis, docking study, and dynamic simulation, are applied to discover a reliable mechanism for colon cancer therapy. Modern pharmacology, involving network pharmacology and molecular study, tends to be a promising scientific approach to uncover interrelationships of phytocompound targets and drugs for colorectal cancer treatment. [15–17] Docking techniques and dynamic simulation were comprehensively employed to clarify stable spatial interactions between the phytocompounds and targets, employing computer technology in the development of cancer drugs. [18–20] Medicinal plants have evolved as an alternative therapy due to less toxicity and cost-effective cancer treatment. [21,22] *Bidens pilosa* (Asteraceae) is one of the edible plants used to medicate various diseases.[23] The abundance of phytoconstituents isolated from EBP consists of polyacetylenes, flavonoids, and phenylpropanoids.[24] The numerous pharmacological effects are detected from EBP for its immunosuppressive, anti-inflammatory, antioxidant, and antiallergic properties.[25] Several biological activities of EBP have been used to treat numerous diseases, for instance, gastritis, asthma, infectious diseases, diabetes, and cancer. [26,27] The anti-cancer properties of EBP against alcohol-induced colorectal cancer remain unclear. This current research, therefore, aims to explore the gastroprotection of EBP through bioinformatic and experimental analysis. The blood alcohol parameters and histological feature were identified in alcoholic rats with different dosages of EBP. The molecular interaction is conducted to verify the potential phytoconstituents of EBP against alcohol-induced colon carcinogenesis as the reference for further clinical application.

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Materials and Methods

Authentication of Plant
Bidens pilosa leaves were obtained from Medan (HPCH+C82, North Sumatera, Indonesia) on April 1, 2023. The plant was classified by an ethnobotanist (State University of Medan, Indonesia). The specimens were assigned the voucher number UNIMED01042023.

Preparation of Plant Extract
Bidens pilosa dried leaves were pulverized by a grinder. The powder was soaked in ethanol (95%) in a 1:10 proportion at 27^0 C. The mixture was stirred three times a week and filtered using Whatman filter paper. The extract was concentrated by a rotavapor at 20^0 C for further analysis.

Preparation of Animal Design
All scientific protocols were assigned the approval number 0453/EPH-FMIPA/2019 by the Ethics Commission of State University of Medan. The ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guideline was applied for the treatment. The experimental animals (mean weight 190 ±10 g; n=6/group) were prepared as the previous study [28]. Thirty male rats were classified into five groups for six weeks, as follows: (a) Group K0 was administered CMC (carboxymethyl cellulose) 0.5%; (b) Group K1 was given orally 30% alcohol (10 ml/kg); (c) Group K2 received 30% alcohol 10 ml/kg + EBP 250 mg/kg; (d) Group K3 was treated with 30% alcohol 10 ml/kg + EBP 500 mg/kg; and (e) Group K4 received orally 30% alcohol 10 ml/kg + EBP 750 mg/kg. On the last experimental period, the animals were anesthetized after fasting overnight (12 h) to harvest the blood and colon for further measurement.

Colon Tissue Analysis
Isolated colons were washed with phosphate–buffered saline. The colon tissues were fixed (10% formalin) for 36 h and dehydrated in graded alcohol (60-95%). The embedded specimens were trimmed into 4-5 µm utilizing a rotary microtome (Chemical Pro Analysis, Indonesia). Haematoxylin and eosin (H&E) method was conducted to assess the colon histoarchitecture under the microscope (Nikon E400, Sanford) at 100x magnification.

Blood Parameter Measurement
Blood specimens were put into EDTA (ethylene diamine tetra acetic acid) tubes to quantify the blood count. The parameters, including mean platelet volume (MPV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and platelets, were determined by a hematological analyzer (Roche Diagnostic, Switzerland).

Statistics
Evaluation of significant difference among groups was performed by analysis of variance, one-way ANOVA, and SPSS 23. This performance is followed by the Duncan's comparison test. Data were presented as mean ± SD. A significant value was estimated at $P < 0.05$.

Construction of Pharmacological Pathways
Bidens pilosa phytoconstituents were taken from the medicinal plants database. The 3D structure of *B. pilosa* compounds was downloaded from the PubChem database. The mechanism pathway between the compounds, alcohol, and the colon diseases target was established using the STRING database ver. 11.5 with the specific target "*Homo sapiens*." The confidence score was set at 0.7. [29] The formatted output was imported into Cytoscape ver. 3.9.1 for understanding the main core target-compounds pathway through betweenness centrality and closeness centrality parameters. The pharmacological pathways were constructed as an underlying mechanism of essential targets.

Molecular Docking Studies
The docking analysis was conducted by Autodock Vina 1.2.4 to evaluate the binding affinities and binding pose between *B. pilosa* compounds and the target. The PDB structures of CASP3, CCL2, EGFR, and STAT3 were taken from the protein database. AutoDock tool ver.15.6 software was applied to format the compounds and protein in the PDBQT file.
The 3D structure targets, comprising CASP3, CCL2, EGFR, and STAT3, were retrieved from the RSCB database (https://www.rcsb.org). The PDBQT file of target proteins and EBP constituents was formatted into the AutoDock tool. The binding affinities of targets and the EBP compounds are estimated utilizing AutoDock ver. 4.2.6. The pose binding of protein-compound complexes was evaluated by Discovery Studio Visualizer Ver. 21.1.0.20298.

Molecular Dynamics Analysis
The simulation of *B. pilosa* compound-protein complexes was described as the root mean square fluctuation (RMSF). [30] The utilization of the CABSflex web server is to estimate the RMSF plot. The prediction of flexibility and stability of protein structure was estimated to perform residual fluctuation in the range of 3.8 Å to 8.0 Å.

Results and Discussion

The accumulation of alcohol leading to DNA damage and ROS production. Alcohol metabolism resulted in toxic compounds containing oxygen radicals, acetaldehyde, and lipid peroxidation.[31] Acetaldehyde, as an endotoxin metabolite from alcohol metabolism, is abundant in the gastrointestinal tract and mediates the alcohol-membrane phospholipid interaction. [32] A previous study demonstrated that the production of aberrant crypt foci was higher in colon tissue. [33] The proliferation of the intestine is accompanied by the production of phosphatidyl ethanol and procarcinogens-N-nitrosamines. [34] Our finding discovered that histopathological damage was found in the K1 group. The disruption of colon histopathology has been characterized through depletion of goblet cells, the damage of neutrophilic infiltrating crypt, lamina propria cell density, and mucosal ulceration.

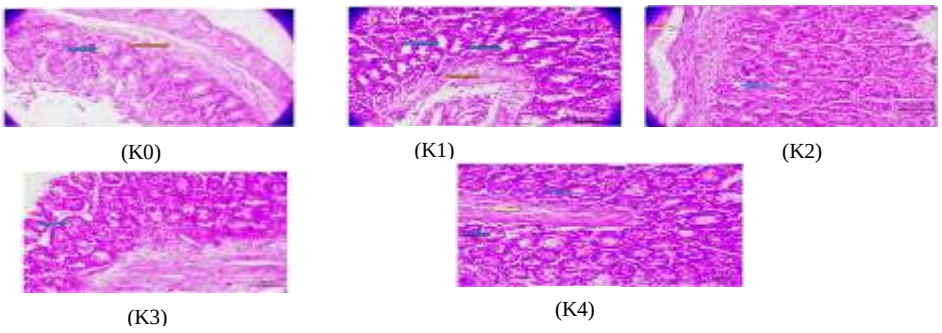

Figure 1: Histoarchitecture changes in the colon by the H&E method: (K0) the control groups CMC 0.5%; (K1) the rat groups fed 30% alcohol 10 ml/kg; (K2) 30% alcohol 10 ml/kg + EBP 250 mg/kg; (K3) 30% alcohol 10 ml/kg + EBP 500 mg/kg; and (K4) 30% alcohol 10 ml/kg + EBP 750 mg/kg. (n= 6, 100x magnification); the yellow arrow: mucosal ulcer; the blue arrow: goblet cell loss.

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(a) (b) (c)

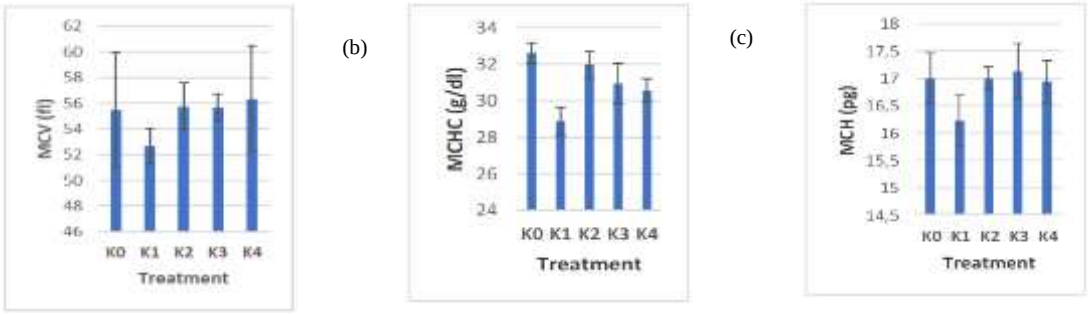

Figure 2: Effect of treatment with EBP on blood parameters (a) MCV-Mean Corpuscular Volume, (b) MCHC- Mean Corpuscular Haemoglobin Concentration, and (c) MCH- Mean Corpuscular Haemoglobin. (K0) the control group CMC 0.5 %; (K1) 30% alcohol 10 ml/kg; (K2) 30% alcohol 10 ml/kg + EBP 250 mg/kg; and (K3) 30% alcohol 10 ml/kg + EBP 500 mg/kg; and (K4) 30% alcohol 10 ml/kg + EBP 750 mg/kg. (n=6, $^{*}p < 0.05$).

(a) (b)

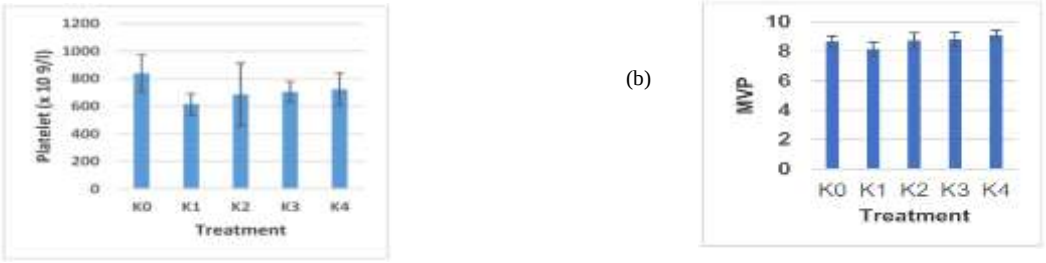

Figure 3: Effect of treatment with EBP (a) Platelet and (b) MVP- Mean platelet Volume. (K0) the control group fed CMC 0.5 %, (K1) 30% alcohol 10 ml/kg, (K2) 30% alcohol 10 ml/kg + EBP 250 mg/kg, and (K3) 30% alcohol 10 ml/Kg + EBP 500 mg/kg, and (K4) 30% alcohol 10 ml/Kg + EBP 750 mg/kg. (n=6, $^{*}p < 0.05$)

The K3 and K4 groups showed that there was a noticeable improvement in the colon tissues (Figure 1**).**

Recent studies have stated that higher levels of phosphatidyl ethanol in blood parameters could be an important biomarker for colorectal cancer screening. [35,36] Additionally, blood alcohol is related to low oxygen tension (2-8%) by promoting cell proliferation and differentiation in disruption of the intestinal barrier. [37] The clinicopathological parameters are used for the indicator of colorectal cancer development, such as platelet count and mean platelet volume.[38] The results verified that the blood alcohol parameter, for example, the level of MCV **(**Figure 2) in the alcohol group (K1) (52.68 ± 1.35), is lower than the K0 group (55.5 ± 4.43). In contrast, the animal treated with EBP at a dose of 750 mg/kg improved the MCV values at 56.36 ± 4.13. According to Figure 3, the alcohol administration decreased the MVP level to 8.15 ± 0.43 compared to the K0 group at 8.64 ± 0.38. Treatment with EBP at a dose of 750 mg/kg significantly ($p < 0.05$) increased the levels of MVP at 9.1 ± 0.3. This study claimed that the EBP supplementation (500 and 750 mg/kg) increased the blood alcohol parameters. The outcome data is similar to the prior evidence that the active phytoconstituents, for instance flavonoids and polyacetylenes, presented remarkable efficacy to suppress oxidative mechanisms and cell proliferation. [39] Oxidative alcohol metabolism contributes to the protein degradation and the activation of post-translational modification (PTMs) in intestinal tight junctions. In the chronic alcohol group, colorectal cancer patients have a higher metastasis rate.[40] Chronic alcohol abuse accelerated the metastasis of CRC progression by promoting arsenic-induced tumor angiogenesis and upregulating the expression of epithelial-mesenchymal transition (EMT) genes.[41] The ameliorative effect of phytocompounds from EBP has been previously reported to suppress signaling pathways in cancer progression.[42] The molecular mechanism of EBP against colorectal carcinogenesis is still unclear. Therefore, these strongly exhibited the potential biological activities from EBP that could stimulate the expression of gene signaling related to apoptosis and proliferation in chronic alcohol-induced colorectal carcinogenesis using bioinformatic studies. [43] Network pharmacological analysis exhibited 71 nodes and 414 edges (Figure 4A**)** that indicated the role of EBP and 5-fluorouracil involved in the therapeutic mechanism of colon cancer. The algorithms from Cytoscape, for instance, betweenness and closeness centrality, were set to refine the core targets. The outcome data identified 12 hub genes for colon cancer treatment, such as UBC, CTNNB1, EGFR, IL6, STAT3, CASP3, PTEN, GRB2, CDH1, CYP2E1, PIK3CA, and VEGFA. The potential of EBP significantly enriched the pathways in colon carcinogenesis therapy, including endometrial cancer, EGFR tyrosine kinase, PD-L1 expression, the TNF signaling pathway, the IL-17 signaling pathway, colon cancer, choline metabolism in cancer, the T and B cell receptor signaling pathway, gastric cancer, the JAK-STAT signaling pathway, and the PD-1 checkpoint pathway in cancer, as listed in Table 1. Based on Figure 4B**,** EBP has a potential as an anti-proliferative and apoptosis that is related to the biological metabolism pathways in colon carcinogenesis caused by alcohol. Our study suggested that the core targets of CASP3, CCL2, EGFR, and STAT3 (**F**igure 5) have become the important keys to inhibit the proliferation and apoptosis of colorectal cancer cells. To clarify the pharmacological pathway, the docking study and RMSF analysis **(**Figure 6**)** were established. The best binding pose of the EBP phytocompounds docked to the CASP3 (Table 2**)** and EGFR (Table 3**)** is linked to the IL-17 signaling pathway. Our finding is similar to the previous study that the enhancement of CASP3 activity has been generated by CD57+ tumor cells. [44] This research claimed that EBP downregulated the expression of the p-STAT3 signaling pathway in order to inhibit apoptosis in colorectal carcinogenesis.[45] The STAT3 signaling pathway enhances the regulation of chemokine CCL2, which tends to be a crucial downstream target to stimulate angiogenesis and tumor growth in CRC drug resistance. [46,47] The previous study had declared the ability of EBP to alleviate the inflamed colon by decreasing TNF-α and IL-1β levels and downregulating MAPK3 and NFκB pathways.[48] These

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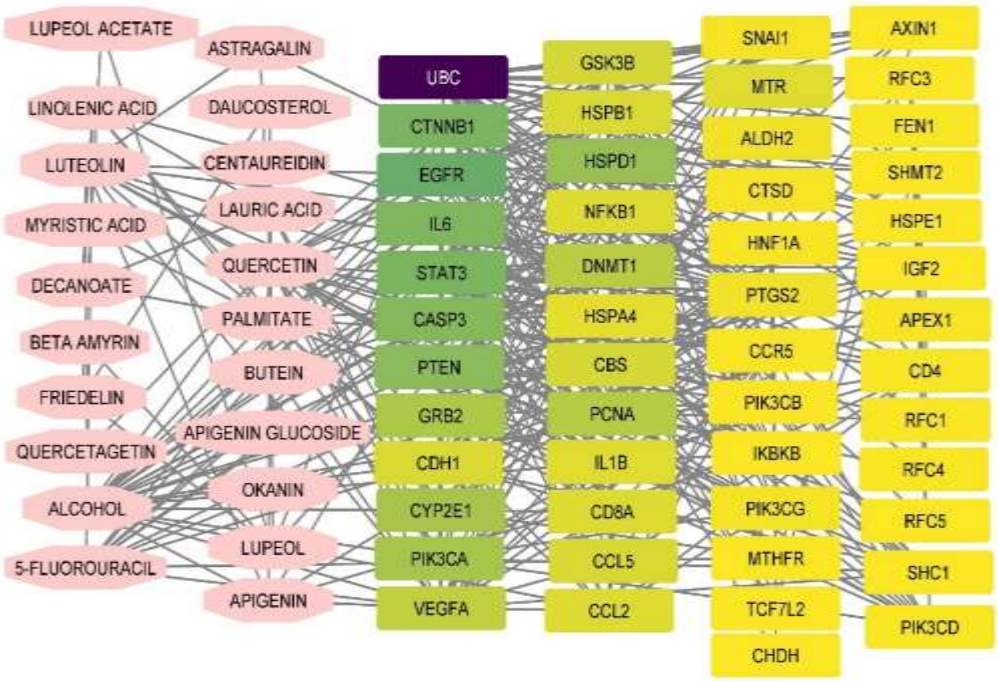

Figure 4A: Key targets of *B. pilosa* against alcoholic colorectal disease (octagon nodes represent candidate compounds of *B. pilosa*).

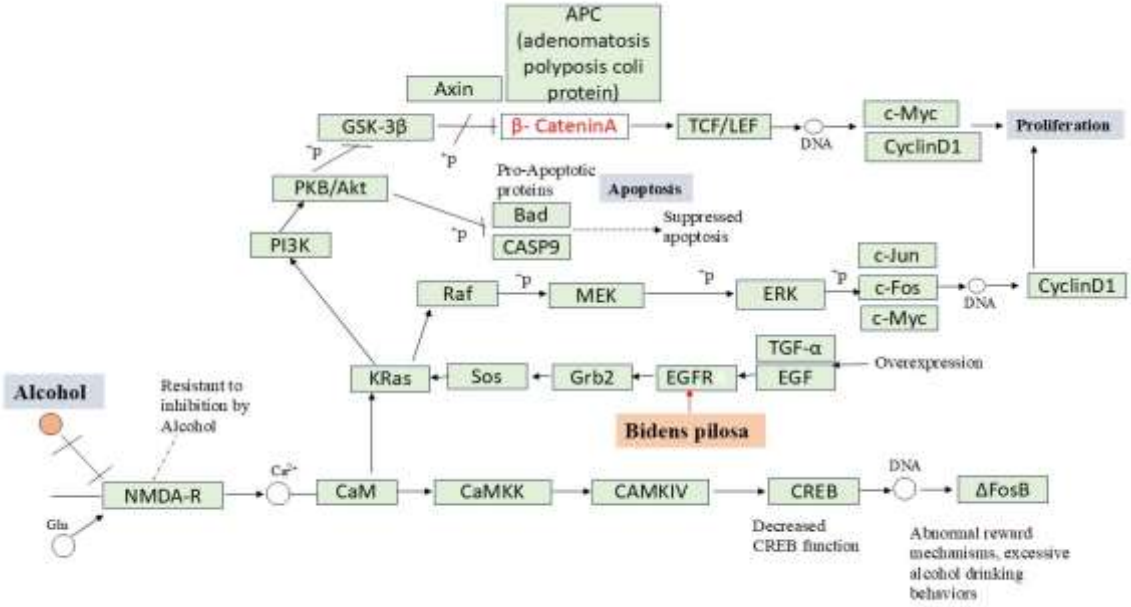

Figure 4B: Key targets of the signaling pathway from *B. pilosa* against alcoholic colorectal disease

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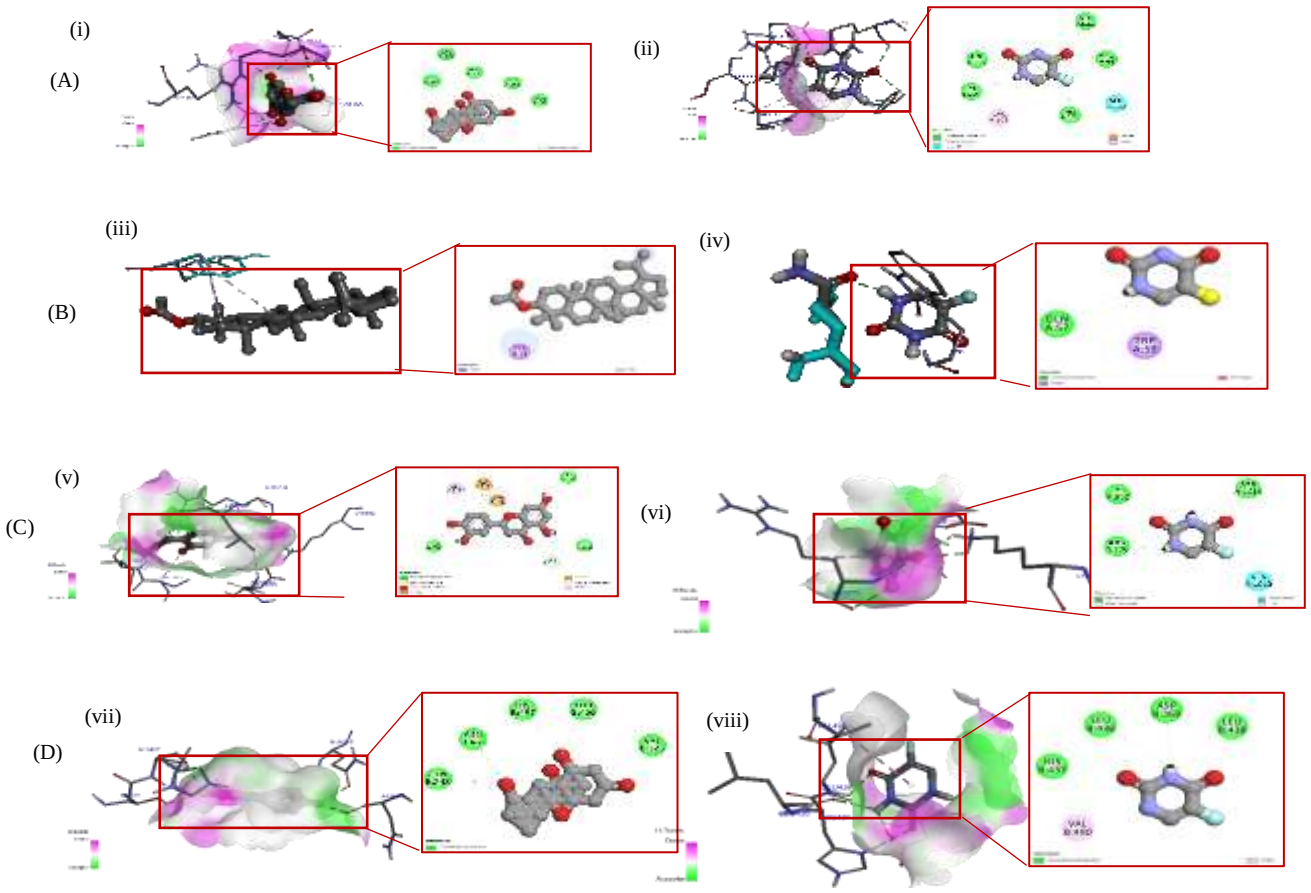

Figure 6: Dynamic Simulation of *B. pilosa* with (A) CASP3 (i) Apigenin (ii) 5-fluorouracil; (B) CCl2 (iii) Lupeol acetate (iv) 5-fluorouracil; (C) EGFR (v) Luteolin (vi) 5-fluorouracil; (D): STAT3 (vii) Apigenin (viii) 5-fluorouracil

Table 1: Enrichment pathways of *Bidens pilosa* in alcohol- induced colorectal injury

Pathway ID	Pathway Description	Count in Gene set	Gene	False Discovery rate
hsa05213	Endometrial cancer	7	PIK3CA, PIK3CB, PIK3CD, CDH1, EGFR, PTEN, GSK3B	6.23e-10
hsa05235	PD-L1 expression and PD-1 checkpoint pathway in cancer	9	PIK3CA, PIK3CB, PIK3CD, PTEN, EGFR, STAT3, NFKB1, CD4, IKBKB	1.09e-11
hsa01521	EGFR tyrosine kinase	8	PIK3CA, PIK3CB, PIK3CD, PTEN, GSK3B, EGFR, STAT3, IL6	1.12e-10
hsa04668	TNF signaling pathway	10	PIK3CA, PIK3CB, PIK3CD, CCL2, CCL5, NFKB1, IKBKB, IL1B, IL6, CASP3	2.36e-12
hsa04660&hsa4662	T and B cell receptor signaling pathway	8	PIK3CA, PIK3CB, PIK3CD, CD8A, NFKB1, GSK3B, CD4, IKBKB	5.54e-10&1.16e-07
hsa04657	IL-17 signaling pathway	7	GSK3B, CASP3, IL6, IL1B, NFKB1, CCL2, IKBKB	9.36e-09
hsa05210	Colorectal cancer	6	PIK3CA, PIK3CB, PIK3CD, EGFR, GSK3B, CASP3	1.50e-07
hsa05231	Choline metabolism in cancer	4	PIK3CA, PIK3CB, PIK3CD, EGFR	0.00015
hsa05226	Gastric cancer	6	PIK3CA, PIK3CB, PIK3CD, EGFR, GSK3B, CDH1	2.66e-06
hsa04630	JAK-STAT signaling pathway	6	PIK3CA, PIK3CB, PIK3CD, EGFR, STAT3, IL6	3.80e-06
hsa05200	Pathway in cancer	13	PIK3CA, PIK3CB, PIK3CD, GSK3B, CDH1, IGF2, CASP3, PTEN, STAT3, EGFR, NFKB1, IL6, IKBKB	2.99e-10

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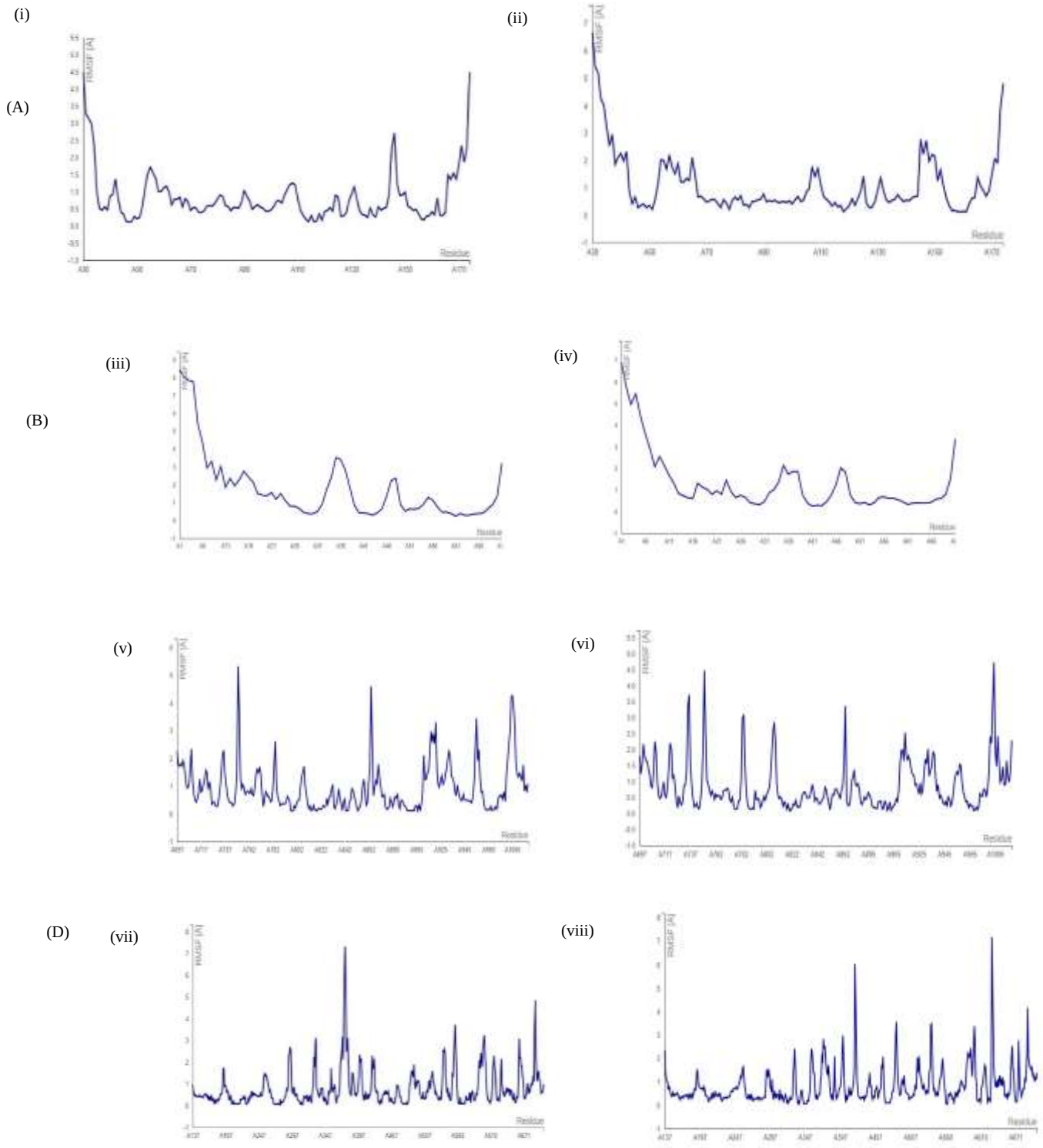

Figure 6: Dynamic Simulation of *B. pilosa* with (A) CASP3 (i) Apigenin (ii) 5-fluorouracil; (B) CCl2 (iii) Lupeol acetate (iv) 5-fluorouracil; (C) EGFR (v) Luteolin (vi) 5-fluorouracil; (D): STAT3 (vii) Apigenin (viii) 5-fluorouracil

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Table 2: Docking and RMSF analysis of the selected compound from *B. pilosa* at the active site of CASP3 and CCL2

No.	Compound	CASP3			CCL2		
		Binding Affinity (kcal/mol)	Hydrogen bond	Hydrophobic Interaction	Binding Affinity (kcal/mol)	Hydrogen Bond	Hydrophobic Interaction
1	Daucosterol	-6.8	Asn208, Trp214, Glu248	Trp206, Trp214, Phe250, Phe256	-6.8	Lys38	Pro8, Thr10, Tyr13
2	Linolenic acid	-4.4	Gly165	Met61, Phe128, Tyr204, Phe256	-3.7	Lys38	Tyr13
3	Lupeol acetate	-7.0	His121, Gly122	Phe256	-7.5	Tyr13	-
4	Apigenin 7-*O*-glucoside	-7.7	Arg64, Gln161, Arg207, ASn208, Ser209, Phe250	Trp206, Arg207	-6.9	Glu50, Cys52	Tyr13
5	Apigenin	-8.5	Arg64, Ser120, His121, Gln161, Tyr204, Arg207	Tyr204, Arg207	-6.5	Lys58, Trp59	Gln57, Lys58, Trp59
6	Butein	-6.3	Tyr204, Asn208, Trp214, Ser251	Trp206, Phe256	-5.9	Tyr13	Tyr13
7	Okanin	-6.5	Arg64, Gln161, Arg207	His121 (salt bridge)	-6.2	Tyr13, Lys38	Tyr13
8	Centaureidin	-6.6	Asn208, Asp211, Trp214, Gln217, Phe250	Trp206, Phe247	-5.8	Phe15, Arg29, Glu50, Cys52	-
9	Luteolin	-7.8	Arg64, Ser120, Gly122, Gln161, Arg207	His121, Tyr204, Arg207	-6.3	Ile5, Ala7, Thr10, Arg29, Thr32, Ser33	Val9
10	Quercetin-3,4′-dimethyl ether-7-*O*-rutinoside	-7.2	Ser63, Ser65, Gly122, Arg207,	His121	-6.5	Asn14, Thr16, Arg24, Thr45, Cys52	Asn14, Lys49
11	Quercetagetin 3,6,3′-trimethyl ether	-6.3	Tyr204, Ser209, Trp214	Phe250	-5.5	Asn14, Arg29, Cys52	-
12	5-fluorouracil	-5.0	Arg64, Gln161, Ser205, Arg207	Arg207 (pi-cation interaction)	-3.9	Lys58, Trp59	-

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Table 3: Docking study and RMSF of the phytocompound from *B. pilosa* at active site of EGFR and STAT3

No.	Compound	EGFR			STAT3		
		Binding Affinity (kcal/mol)	Hydrogen bond	Hydrophobic Interaction	Binding Affinity (kcal/mol)	Hydrogen bond	Hydrophobic Interaction
1	Daucosterol	-8.3	Glu762, Leu788, Thr790, Thr854, Asp855	Leu718, Leu792, Leu844	-8.4	Gln248, Gln326, Thr456, His457	Lys233, Asn315, Lys318
2	Linolenic acid	-5.5	Leu747, Asn756	Phe723, Leu747, Ala755, Glu758, Ile759, Leu858	-5.5	Ser372, Ser381	Lys370, Asp371, Ala376, Leu438, Val490
3	Lupeol acetate	-8.8	-	Leu777, Leu788, Arg841, Leu844, Asp855	-7.5	-	Gln247, Ile258, Arg325
4	Apigenin 7-*O*-glucoside	-8.7	Glu762, Leu788, Thr854, Asp855	Leu718, Val726, Lys745	-9.2	Lys370, Asp371, Gly373, Asp374, Leu378, Ser381, Leu438, Thr440	Leu438, Lys488
5	Apigenin	-8.7	Leu747	Phe723, Leu747, Ala755, Ile759, Val786	-10.4	Val323, Thr456, His457	Lys244, Val 323, Leu459, Pro487
6	Butein	-8.1	Leu703, Val774, Arg776, Lys852, Ile1018	Arg776, Leu778	-8.3	Asp369, His437, Leu438, Thr440, Glu455	Lys370, Asp371, Leu438
7	Okanin	-8.2	Val774, Arg776, Lys852	Gln791, Ile1018	-8.4	Ala377, Ser381, Leu436, His437, Leu438, Thr440, Glu455	Lys370, Asp371, Leu438
8	Centaureidin	-8.2	Arg776, Asp1014, Ile1018	Asp1014	-9.3	Lys370, Asp371, Ala377, Ser381, Thr440	Asp371, Leu438
9	Luteolin	-9.0	Val774, Arg776, Leu778, Lys852, Ile1018	Arg776, Leu778, Gln791	-9.1	Lys370, Asp371, Ala377, Ser381, Arg382, Thr440	Asp371, Leu438, Val490
10	Quercetin-3,4′-dimethyl ether-7-*O*-rutinoside	-8.3	Leu703, Arg705, Arg776, Asp1014, Ile1018	Arg776, Leu778, Leu1017	-8.7	Asp369, Lys370, Ser372, Ala377, Ser381, Leu438, Thr440, Asn491	Leu438, Lys488
11	Quercetagetin 3,6,3′-trimethyl ether	-9.1	Val774, Arg776, Lys852, Ala1013, Ile1018	Arg776, leu778, Gln791	-8.6	Lys370, Ser381, Leu438, Thr440	Leu438, Val490
12	5-fluorouracil	-5.1	Arg776, Lys852, Ala1013, Asp1014	Asp1014	-5.2	Asp369, Ser372, His437, Leu438	-

Findings demonstrated the preventive effects of EBP against alcohol-induced colorectal cancer through improvement of the alcoholic colon damage and enhancement of the proliferation and apoptosis signaling pathway.

Conclusion

In summary, the EBP plays a gastroprotective effect against alcohol-induced colorectal injury to ameliorate the colon histological change and improve the level of blood alcohol parameters. The bioinformatic preliminary findings clarified that the phytocompound of EBP has anti-cancer properties to regulate proliferative and apoptosis pathways, particularly CASP3, EGFR, CCL2, and STAT3 in alcohol-induced colon carcinogenesis. This study contributes to a better understanding of the chemopreventive effect of EBP against colon carcinogenesis for further preclinical studies.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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