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Original Article

INVESTIGATING THE TOXICITY OF BETALAIN COMPOUNDS: *IN SILICO* ANALYSIS AND *IN VIVO* PREDICTIONS FOR STANDARDIZED *BETA VULGARIS* L. EXTRACT

SONY EKA NUGRAHA[1*], JANE MELITA KELIAT[2], MARIANNE[3], RONY ABDI SYAHPUTRA[3]

[1]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia. [2]Faculty of Vocational Study, Universitas Sumatera Utara, Medan, Indonesia. [3]Department of Pharmacology, Faculty of Pharmacy, Department of Pharmacology, Universitas Sumatera Utara, Medan, Indonesia

***Corresponding author: Sony Eka Nugraha; *Email: sonyekanugraha@usu.ac.id**

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ABSTRACT

Objective: Extensive research has been conducted on beetroot's antioxidant, hematoprotective, and cardioprotective properties. However, there currently needs to be more available evidence pertaining to the toxicity assessment of the extract. The toxicity assessment was conducted using both *in silico* and *in vivo* methods. Prior to testing, the extracts were standardized in accordance with the guidelines set by the Indonesian Food Drug Authority (BPOM), which is the regulatory authority for food and drugs in Indonesia.

Methods: The experimental subjects consisted of 25 male Wistar rats in good health, weighing between 150 and 170 grams. These rats were separated into five groups, each including five rats. Group 1 will serve as the control group, while groups 2 through 5 will be designated as the treatment groups. The analysis of chemical toxicity was conducted using pK-CSM, SwissADME, and Pro-Tox II methodologies.

Results: The results indicated that the standardized ethanol extract contained 4.341% water, 3.67 % total ash, and 1.53 % acid-insoluble ash. Lead (Pb) and cadmium (Cd) were absent at a concentration of 0 parts per million (ppm). Subsequently, the total plate count and yeast mould count were 0.47 5 x 10-4 (CFU/g) and a of 0.382 x 10-4 (CFU/g) respectively. This finding implies that the extract meets BPOM requirement. This study also measured the betalain content of red beetroot, yielding a total concentration of 11.34 0.37 mg/100 gram of sample. Haematological experiments showed that beetroot extract affected rat blood haematology. Compared to the control group, rats given the extract had higher red blood cell and platelet counts. Additionally, the Insilico toxicity test conducted on the active component derived from beetroot revealed LD50 of the compounds ranged from 305 mg/kg so that were categorized into classes IV and presence of hepatotoxic potential. During the *in vivo* experiment, there has been a notable rise in hepatic and renal parameters. Furthermore, one mortality event occurred in the test subject at a 5,000 mg/kg body weight dosage.

Conclusion: Single oral administration of the extract at a dose larger than 5,000 mg per kilogram of body weight does not result in lethal effects, however showed potential toxicity to the liver.

Keywords: *Beta vulgaris* L., *In silico*, *In vivo*, Toxicity

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INTRODUCTION

Beetroot (*Beta vulgaris* L.), also known as beets, is a highly pigmented and nutrient-dense vegetable that is classified as a member of the Chenopodiaceae family [1]. The distinctively dark crimson hue of beetroot makes it an aesthetically pleasing addition to a variety of culinary preparations while also providing numerous health benefits. Beets are an excellent source of essential vitamins and minerals, including vitamin C, potassium, and manganese [2]. In addition, beetroot contained high dietary fiber, making it a significant contributor to overall health and wellness. Moreover, beetroot is renowned for its high nitrate content, which has been associated with improved cardiovascular health and enhanced physical performance during exercise [3].

Beetroot, whether consumed in raw, roasted, pickled, or juiced form, has a distinct and gratifying earthy taste. This characteristic renders it a versatile and nutritionally dense choice for persons who prioritize their well-being and aim to include a nourishing vegetable in their dietary regimen [4]. Furthermore, it is commonly recognized that beets contained high antioxidant. Antioxidant play role in exhibit anti-inflammatory qualities, which may contribute to the alleviation of inflammation and the control of specific chronic illnesses [5]. The inclusion of beetroot in one's dietary regimen can be easily achieved by its use in various culinary preparations such as salads, smoothies, or even as a flavorful complement when barbecued [4]. Given its notable assortment of health-enhancing characteristics, beetroot is a commendable inclusion in a well-rounded and nourishing dietary regimen. Prior studies have discovered the presence of cardioprotective and hematoprotective characteristics in beetroot (*Beta Vulgaris* L.), which have exhibited promising therapeutic qualities as both pharmacological agents and dietary supplements [6, 7]. Beetroot is a consumable material that has a purple-red coloration. The observed purple-red hue exhibited by beets can be attributed to the existence of betalain pigment, which is a composite of betacyanin, a purple pigment, and betaxanthin, a yellow pigment [8]. Nevertheless, there remains a scarcity of empirical data to substantiate the safety assertions stated regarding beetroot. Hence, it is essential to carry out further animal tests to ascertain the existence of any potential toxicological impacts, thereby guaranteeing the safety and suitability of its utilization. The assessment of toxicity can be conducted using both *in vitro* and *in vivo* techniques.

The *in vivo* study outcomes of the current study are anticipated to provide information regarding the LD50 and the administration of appropriate doses and identify detrimental manifestations. The acute toxicity test is a preclinical evaluation in which chemical substances are evaluated on experimental animals prior to administration to humans. The acute toxicity test is a method for identifying and evaluating the toxicological effects that manifest immediately after the introduction of a chemical, either through a single dose or multiple doses administered within a maximum of 24 h [9].

Throughout history, the predominant approach for toxicological evaluations has involved the utilization of *in vivo* experiments, where in animals are subjected to the administration of substances for the goal of testing. Furthermore, the vast variety of chemical compounds produced by *Beta vulgaris* L. makes it difficult to assess their toxicity using only experimental methods. Hence, it is imperative to give precedence to advancing alternative methodologies that exhibit swiftness, cost-effectiveness, and adherence to ethical principles. Computer-based toxicity prediction

models, known as *in silico* models, have emerged as feasible alternatives to traditional approaches for assessing toxicity [10]. The computational algorithms employed in this study leverage molecular structures and physicochemical features to make predictions regarding its toxicity. By using available data and knowledge resources, *in silico* approaches facilitate the prioritization and assessment of chemical compounds for subsequent investigation. The existence of this research gap highlights the necessity for an integrated approach that combines *in vivo* and *in silico* approaches to evaluate the toxicity of these compounds effectively. By integrating the advantages of both techniques, a more resilient and streamlined framework for predicting toxicity can be developed.

MATERIALS AND METHODS

Reagents and chemical

The tools used are glassware, hot plate (Fisons), desiccator, cage, label paper, pH paper, filter paper, Whatmann No.42 filter paper, analytical balance (Boeco), light microscope (Boecp), infusion pot, set of surgical tools, thermometer, and scales. The chemical used were Ethanol (BrataChem), Methanol (BrataChem) Water pro-injection (Sigma Aldrich), Hematoxylin and Eosin (Sigma Aldrich), AST kit (Roche), ALT kit (Roche), Urea kit (Roche), and Creatinine kit (Roche), buffered formalin (bratachem), and hematoxylin and eosin.

Preparation and extraction

Preparation of ethanol extract of beet tubers was carried out by maceration with 96% ethanol solvent and then evaporated with a rotary evaporator at 40 °C until a concentrated is obtained [8].

Extract standardization

Standardization of extracts includes specific and nonspecific parameters carried out according to the Indonesian Food Drug Authority (BPOM), including organoleptic parameters such as color, smell, shape, taste and nonspecific parameters such as water content, total ash content, acid insoluble ash content, Pb contamination, Cd contamination, total plate count, mold and yeast count [11].

Betacyanin content analysis

The photometric quantification of total betalains was determined standard method [12]. The solutions were diluted using McIlvaine buffer (having a pH of 6.5 and consisting of citrate-phosphate in a 1:10 proportion). The calculation for total betalains was conducted as outlined below:

$$B\ [mg/g] = \frac{A \times DF \times MW \times V}{\epsilon \times L}$$

Where: A = value at maximum absorption (534 for betacyanins and 480 for betaxanthins) at 600 nm, DF = dilution factor, MW = molecular weight (550 g/mol for BC and 308 g/mol for BX), V = volume of the solution (1000 ml), Є = molar extinction coefficient (60,000 L/mol cm for betacyanins and 48,000 L/mol cm for betaxanthins), and L = length of the reading cell (1 cm). The quantification of betacyanins and betaxanthins was conducted individually, and afterward, the respective measurements were combined to establish the overall betalain concentration. The measurements were conducted on three occasions, and the outcomes were reported as milligrams per 100 grams of powder.

In silico tools

The tools used in this research are hardware in the form of a set of HP Core i3 64-bit specifications and 1 TB hard disk as well as software and operating system software. Windows 10, Pubchem, pK-CSM Tools, SwissAdme, and Pro-Tox II.

Preparation of compound for *in silico* toxicity prediction

The preparation of each compound to obtain Canonical SMILES was carried out using the Pubchem website (https://pubchem.ncbi.nlm.nih.gov/)

Toxicity prediction of compound with pK-CSM tools

Prediction of compound toxicity using pK-CSM Tools via http://biosig.unimelb.edu.au/pkcsm/prediction, is done by entering Canonical SMILES, then pressing ADMET to get absorption analysis results distribution (VDss, Fraction unbound, BBB permeability, and CNS permeability); metabolism and toxicity [13].

Toxicity prediction of compound with Pro-Tox II

Prediction of compound toxicity with Pro-Tox II is accessed via https://tox-new.charite.de/protox_II/, then press Tox Prediction and enter Canonical SMILES, tick all toxicity parameters, then Start Tox-Prediction to get the results of the toxicity analysis of the compound (LD50, Hepatotoxicity, Carcinogenicity, Immunotoxicity, Mutagenicity, Cytotoxicity, AhR, AR, AR-LBD, Aromatase, ER, ER-LBD, PPAR-Gamma, nrf2/ARE, HSE, MMP, Phosphoprotein tumor suppressor, and ATAD 5) [14].

Oral acute toxicity prediction

The number animals used were 25 healthy male rats weighing 150-170 g. divided into 5 groups, each group consisting of 5 rats. Group 1 as control, and groups 2-5 as treatment groups. Group division as follows:

Group 1: Control, given a suspension solution of Na-CMC 0.5% kgbw

Group 2: Treatment, given beetroot extract at a dose of 500 mg/kgbw.

Group 3: Treatment, given beetroot extract at a dose of 1000 mg/kgbw.

Group 4: Treatment, given beetroot extract at a dose of 2000 mg/kgbw

Group 5: Treatment, given beetroot extract at a dose of 5000 mg/kgbw.

The test preparation was given to the test animals by oral, and it was only given once during the test time. The test animals were first fed for 3-4 h while still given a drink. From each group taken randomly, the toxic effects that occur were observed compared with the control group. The observation time was 5 min, 10 min, 15 min, 30 min, 60 min, 120 min, 180 min, 240 min. The total observation time was 4 h and after that for 14 d. Rats were observed and LD50 was determined to measure the level of toxicity by looking at the number of dead rats. Subsequently, blood was taken through the inferior vena cava [15]. Liver and kidney health biomarkers were measured and organ histopathological images were taken use standard procedure [16-18].

RESULTS AND DISCUSSION

Extract standardization

Examination results standardization specific parameters, including organoleptic shows in the table 1.

Table 1:Organoleptic parameters of the extract

No	Parameter	Results
1	Color	Blackish Purple
2	Smell	Typical
3	Form	Thick
4	Flavor	Bit Bitter

Standardization inspection results for specific parameters, include water content, total ash content, acid insoluble ash content, Pb contamination, Cd contamination, total plate number, and yeast mold number, are shown in the table 2.

The sample analysis indicates a water content of 4.341%, signifying a relatively low moisture level, a high-water content can lead to the growth of fungi and mold, which can damage the sample. Additionally, the total ash content of 3.67% reflects the mineral constituents, while the acid-insoluble ash content of 1.53% suggests minimal contamination with substances like sand another inorganic compound. Furthermore, the sample exhibits no detectable

contamination of toxic heavy metals, with both lead (Pb) and cadmium (Cd) being 0 ppm; this aligns with safety standards, emphasizing the product's safety regarding these heavy metals. The microbial analysis, including total plate fig. 0.47 5 x 10^{-4}(CFU/g) and yeast mold numbers 0.382 x 10^{-4}(CFU/g) indicated a low microbial presence, suggesting that the sample has been preserved or stored appropriately. Collectively, these results highlight a sample with commendable purity, minimal contaminants, and a favorable profile for safety and stability, though comparison with industry benchmarks is essential for comprehensive understanding [19].

Betalain content

The present investigation involved the extraction of betalains from red beet, resulting in an overall concentration of 11.34±0.37 mg per 100 grams of the sample. The betalain calculation results are shown in table 3.

Table 2: Extract standardization results of nonspecific parameters of beetroot extract

No	Test parameters	Analysis results
1	Water content	4.341 %
2	Total Ash Content	3.67 %
3	Acid Insoluble Ash Content	1.53 %
4	Pb contamination	0 ppm
5	Cd contamination	0 ppm
6	Total Plate Figures	0.475 x 10^{-4}(CFU/g)
7	Yeast Mold Numbers	0.382 x 10^{-4}(CFU/g)

Table 3: Betalain content analysis

Betacyanin (mg/100 g)	Betaxanthin (mg/100 g)	Total betalain (mg/100 g)
6.32±0.27	4.02±0.1	11.34±0.37

All values are mean±SD values (Number of experiment, n=3)

Specifically, the betacyanin pigments, which characterized a red-violet hue, were found to be present at a concentration of 6.32±0.27 mg/100 g, while the betaxanthin pigments, characterized by their yellow coloration, were detected at a concentration of 4.02±0.1 mg/100 g. The observed correlation between the elevated levels of Betacyanin and the distinct crimson coloration exhibited by beets implies the predominant occurrence of this pigment. The observed discrepancies in betalain contents among various research may be attributed to variations in extraction methodologies, environmental conditions, or the specific cultivars of beets utilized [20]. The identified antioxidant of betalains highlights the potential of red beet as a functional food ingredient or natural colorant and potential medicine. Antioxidants play a crucial role in cellular protection by stabilizing and inhibiting the detrimental effects of reactive oxygen species (ROS). This underscores the importance of antioxidants in the preservation of cellular well-being and illness prevention [21].

Biochemical marker for rat liver and Kidney Health

In this study, AST levels were examined from rat blood. The results of the levels obtained can be seen in table 4.

Table 4: Value of biochemical marker for liver dan kidney health

No.	Treatment group	Mean AST (U/l)	Mean ALT (U/l)	Mean creatinine (mg/dl)	Mean Urea (mg/dl)
1.	Group 1	172.81±8.21	61.44±6.72	0.23±0.01	50.4±2.83
2.	Group 2	176.11±8.52	66.32±9.10	0.23±0.01	54.4±2.47
3.	Group 3	219.92±17.25	84.08±4.66	0.25±0.01	60.4±3.4
4.	Group 4	213.8±17.38	89.34±11.62	0.24±0.02	66±3.33
5.	Group 5	243.21±28.41	143.62±12.14	0.22±0.01	65.4±6.74

All values are mean±SD values (Number of experiment, n= 5)

In the examination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels across the treatment groups, it was observed that groups Group 1 and Group 2 exhibited generally comparable and decreased levels of both hepatic enzymes in comparison to the remaining groups. It is noteworthy that Group 5 exhibited an increased aspartate aminotransferase (AST) level of 243.21 U/l, indicating a possible hepatic abnormality. The observation of equal standard deviations in Group 1 and Group 2 for both enzymes emphasize the consistency within these groups, whereas Group 3 and Group 4 exhibit slightly greater variability in ALT. The specific characteristics of the treatments and potential factors that may influence the results are now undisclosed. However, the significantly elevated AST level seen in Group 5 highlights the urgency of doing additional research and considering appropriate medical intervention.

Upon analysis of creatinine and urea levels, it is observed that the average creatinine readings exhibit negligible fluctuations among the different groups, with a range of 0.22 mg/dl to 0.25 mg/dl. This indicates a relatively uniform renal function in terms of creatinine clearance. In contrast, there is a noticeable increase in urea levels from 50.4 mg/dl to 66 mg/dl, followed by a minor decrease to 65.4 mg/dl in the final group (Group 5). Nevertheless, it is important to acknowledge the heightened variability observed in the aforementioned group, as evidenced by the elevated standard deviation for urea. In contrast to the greater variability exhibited by urea values, creatinine values among various groups are more similar. This disparity suggests that factors other than renal function, such as protein ingestion or catabolism, may be responsible for the observed variations in urea levels [22].

The test results in the *in vivo* test showed that a dose of 5000 mg/kgbw showed potential toxicity to the liver. This is in line with the results of an *in silico* study test where the prediction of betacyanin toxicity, which is the group of compounds most abundantly contained in beetroot, shows the results of toxicity to the liver. The number of deaths in this acute toxicity test showed quite good results, where only 1 rat died at a dose of 5000 mg/kgbw. These results still need to be explored further by conducting subchronic and chronic toxicity studies to see the safety profile of the ethanol extract of beetroot.

Results of haematological profile

Table 5: Analysis of rats hematology profile

Parameter	Groups (mean±SD)				
	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**
WBC (10^3/ul)	5±2.06	6±3.32	5±3.65	6±3.65	6.12±2.9
RBC (10^6/ul)	10.022±2.11	12.22±3.21	12.32±1.63	13.32±3.41	13.3±2.45
HGB (g/dl)	11.45±1.51	12.31±3.11	11.36±3.11	12.23±2.21	11.14±3.21
HCT (%)	48.2±6.53	49.9±5.44	54.6±3.50	53.7±5.23	53.14±2.91
MCV (fl)	45.21±5.12	51.23±4.31	50.23±4.12	48.23±1.44	53.5±1.31
MCH (pg)	10.48±2.74	12.22±3.78	13.23±1.89	14.34±3.11	12.54±2.89
MCHC (g/dl)	33.23±4.21	31.34±6.25	34.32±3.43	35.34±4.13	34.67±2.16
PLT (10^3/ul)	1082.4±201.21	1189.9±219.32	1221.2±149.34	1345.2±313.31	1321.4±14.37
NEUs (%)	7.8±3.56	10.8±3.89	10.3±3.76	11.8±3.22	15.6±4.13
LYM P (%)	72.7±8.23	68.2±8.34	74.3±5.34	77.3±7.12	65.4±4.17
MONO (%)	8.3±3.41	7.7±4.17	6.8±3.88	6.8±2.29	7.2±3.83
EOS (%)	1.4±0.2	1.5±0.3	1.5±0.4	1.5±0.2	1.4±0.3
BAS (%)	7.3±3.37	6.7±2.45	7.7±3.22	7.0±4.22	9±3.23

All values are mean±SD values (Number of experiment, n= 5)

The results of the hematological tests showed that beetroot extract had an effect on the hematological changes in rat blood. There was a change in the profile of the red blood cells and platelets of rat; the blood profile in the rats given the extract had a higher value than the normal rat. This is in line with Nugraha's research that supplementation with beetroot extract can improve platelet profiles and red blood cell profiles [6, 7].

Histological observation

The results of observing the histological picture of rat liver cells can be seen in fig. 1

Fig. 1 shows that inflammatory cell infiltration was not found in the control group treatment. At the administration of ethanol extract doses of 500, 1000, 2000 and 5000 mg/kg BW, fatty degeneration and hydrophic degeneration were not found. Fatty degeneration is characterized by the presence of vacuoles that vary in size and in severe cases, push the nucleus to the edge [23]. Hydrophic degeneration is a reversible cell injury with intracellular accumulation that is more severe with albumin degeneration.

The results of observations of the histological appearance of the rats kidney organs can be seen in fig. 2

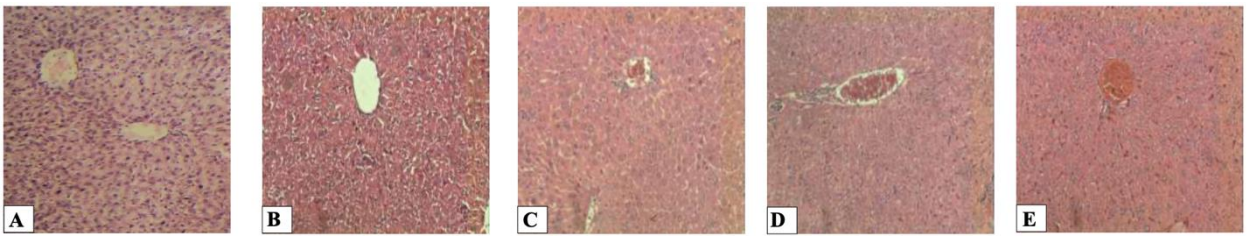

Fig. 1: Histological picture of rat liver tissue 400x (A). Control, (B). 500 mg/kgbw beetroot extract., (C). 1000 mg/kgbw beetroot (D). 2000 mg/kgbw beetroot, (E). 5000 mg/kgbw beetroot

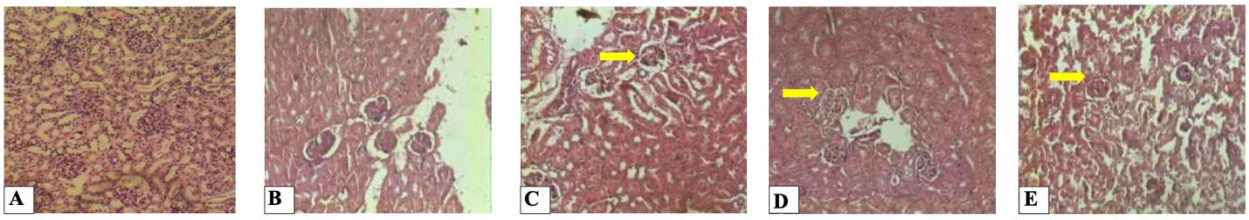

Fig. 2: Histological picture of rat kidney tissue 400x (A). Control, (B). 500 mg/kgbw beetroot extract., (C). 1000 mg/kgbw beetroot, (D). 2000 mg/kgbw beetroot, (E). 5000 mg/kgbw beetroot

Fig. 2 shows that the histological picture of the kidneys in the control group and 500 mg. kgBW were still normal, there was no increase in mesangial cell proliferation in the glomerulus and Bowman's capsule, which was still clearly visible, whereas in the treatment group, the doses were 1000, 2000 and 5000 mg/kg BW it has begun to show an increase in mesangial cell proliferation (yellow arrow) but no glomerular tissue hypertrophy has been seen.

Toxicity prediction of compounds

Betacyanin is a class of active compounds from beetroot which is mostly contained in beetroot. The main structure of the main basic structural units, namely the aglycones betanidine and isobetanidine. The results of Asra's research in 2020 stated that the average percentage of betacyanin levels in the red beetroot extract (*Beta vulgaris* L.) was 98.6474 %±0.584080 [24]. So that the toxicity test of insilico in this study used the active compound betacyanin. Toxicity profile testing and ADME using HP Core i3 64-bit Laptop equipment, Chemdraw application, pKCSM online tool application (http://biosig.unimelb.edu.au/pkcsm/) and Protox application online tool (https://tox-new.charite.de/protox_II/). The results of ADME and toxicity profiling can be seen in table 6-8.

Table 6: Profile ADME of betacyanin

Property	Model name	Predicted value of betacyanin	Unit
Absorption	Water Solubility	-2.866	Numeric (log mol/l)
Distribution	VDss (human)	-1.705	Numeric (log L/kg)
Metabolism	CYP3A4 Substrate	No	Categorical (Yes/No)
Excretion	Total Clearance	0.216	Numeric (log ml/min/kg)

Computational techniques can be employed to evaluate, simulate, or anticipate chemical toxicity, hence facilitating the assessment of pharmacokinetic activity and toxicity [25]. The PK-CSM model has the capability to forecast the Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) features of a given chemical, as indicated by reference [26]. The prediction outcomes are presented in table 6, indicating that Betacyanin exhibits a relatively low water solubility of -2.866 log mol/l. This finding suggests that the oral absorption process may encounter challenges due to the reduced solubility of Betacyanin. The primary localization of Betacyanin within the plasma, as indicated by the computed volume of distribution (VDss) of -1.705 log L/kg, suggests limited diffusion to different organs. The prediction of compound distribution encompasses the consideration of characteristics related to the volume of distribution at steady state (VDss). According to He in 2019, a low VDss value is defined as being below 0.71 L/kg (log VDss<-0.15), whereas a high VDss value is defined as being over 2.81 L/kg (log VDss>0.45 [21]. The acquired distribution prediction findings suggested that the chemicals in question had a low distribution, as evidenced by their VDss values being less than 0.7. It is important to highlight that this chemical exhibits a lack of metabolic activity through the key hepatic enzyme CYP3A4 [22], hence reducing the potential for medication interactions facilitated by this specific metabolic route. Based on the observed overall clearance rate of 0.216 log ml/min/kg, it can be deduced that Betacyanin demonstrates a modest level of removal from the human body. The predictions offer significant insights into the activity of Betacyanin inside biological systems and its potential therapeutic applications.

Table 7: Prediction of betacyanin toxicity (pKCSM)

Property	Model name	Predicted value of betacyanin	Unit
Toxicity	AMES toxicity	No	Numeric (log ml/min/kg)
Toxicity	Max. tolerated dose (human)	0.678	Categorical (Yes/No)
Toxicity	hERG I inhibitor	No	Categorical (Yes/No)
Toxicity	hERG II inhibitor	No	Numeric (log mg/kg/day)
Toxicity	Oral Rat Acute Toxicity (LD50)	2.471	Categorical (Yes/No)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	3.652	Categorical (Yes/No)
Toxicity	Hepatotoxicity	Yes	Numeric (mol/kg)
Toxicity	Skin Sensitisation	No	Numeric (log mg/kg_bw/day)
Toxicity	*T. Pyriformis* toxicity	0.285	Categorical (Yes/No)
Toxicity	Minnow toxicity	8.496	Categorical (Yes/No)

Table 8: Betacyanin toxicity class (protox online)

No.	Parameters	Betacyanin
1.	Predicted LD 50	305 mg/kg
2.	Predicted toxicity class	Class 4
3.	Average similarity	55.2%
4.	Prediction accuracy	67,38%

Pro-Tox II has advantages such as predicting the level of oral toxicity, organ toxicity (hepatotoxicity), toxicological endpoints (such as mutagenicity, carcinogenicity, cytotoxicity, and immunotoxicity), toxicity pathways, and target toxicity, thereby demonstrating the possible molecular mechanisms behind the response [27]. Toxicity class is defined according to the GHS (Globally Harmonized System) classification system, which is categorized into six classes. Class I (LD50 5), class II (5<LD50 50), class III (50<LD50 300), class IV (300<LD50 2000), class V (2000<LD50 ≤ 5000), and class VI (LD50>5000). The higher the LD50 value, the lower the toxicity [28]. Based on table 8, the LD50 of the compounds ranged from 305 mg/kg so they were categorized into classes IV. The results of the study show that the betacyanin toxicity class is class 4 which is included in the dangerous class. In the hepatotoxicity parameter, it is indicated that Betacyanin can cause hepatotoxic. Meanwhile, in the AMES, hERG I inhibitor, hERG II inhibitor and Skin Sensitization toxicity were not found, so it is declared that Betacyanin showed potential toxicity in hepatic organ.

CONCLUSION

Invivo toxicity test revealed that *Beta vulgaris* L. extract in single oral administration of the extract at a dose larger than 5,000 mg/kgbw did not result in lethal effects; however, it showed potential toxicity to the liver. The betacyanin compound *Beta vulgaris* L. has an LD50 ranging from 305 mg/kg so that it is categorized into toxicity classes IV, and it is also indicate hepatotoxicity.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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