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Acute Toxicity Ethanol Extract of Bidara Leaves (*Ziziphus spina-christi* L.) Against Liver and Kidney Function of White Rats

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A B S T R A C T

Bidara plants have the efficacy as antioxidants, anti-inflammatory, antimicrobial, anti- fungal and prevents tumours. Bidara is efficacious to protect human DNA cells caused by damage from actinic radiation. This study aims to explore the acute toxicity test of the ethanol extract of Bidara leaves using white mice as experimental animals. A total of 30 white rats (Rattus norvegicus) Wistar strain obtained from the Eureka Research Laboratory (Palembang, Indonesia) weighing between 200 - 250 grams. After one week of adaptation, the mice were randomly divided into the following six groups, each containing five animals: Normal control group and Bidara extract group (50mg/kg BW; 150 mg/kg BW; 450 mg/kg BW; 1350 mg/kg BW; and 4050 mg/kg BW). This study shows that the extract of Bidara leaves has a relatively high toxic dose, namely at a dose of 4050 mg/kg BW. Bidara leaf extract at doses below 1500 mg/kg BW, shows no toxic effect on the liver. In conclusion, bidara leaf extract has a toxic dose above 4000 mg/kg BW in Wistar white rats.

1. Introduction

Bidara plants contain phenolics and flavonoids which are rich in benefits. Phenolic compounds are compounds that have an aromatic ring with one or more hydroxy groups, compounds derived from plants that have the same characteristics, namely aromatic rings containing one or more hydroxyl groups. Bidara plants, which are rich in phenolic class compounds, have the efficacy, among others, as antioxidants, anti-inflammatory, antimicrobial, anti-fungal and prevent tumors. Bidara is efficacious to protect human DNA cells caused by damage from actinic radiation. Although there have been many studies on the chemical content of Bidara leaves, the clinical use of Bidara leaves is still based on empirical evidence so it is still necessary to determine the pharmacological properties and test the toxicity of the extract.[1-4] Toxicity research needs to be carried out to protect the public from possible adverse effects. As a first step to determine the toxic potential of a substance, a toxicity test is carried out. The acute toxicity test is a procedure designed to determine the median lethal dose of a substance and its possible mechanism of action and target organs. In simple terms, toxicity can be defined as the ability of a compound that can cause harmful effects or deviate from the biological mechanism in an organism. The toxic effects of drugs can be observed from the morphology and histology of the liver, because of their central role in the metabolism of all drugs and foreign substances that

enter the body. The liver will change the structure of the lipophilic drug to become hydrophilic so that it is quickly excreted from the body through urine or bile. The liver becomes the target organ for several reasons, such as a large part of the toxic substances enter the body through the gastrointestinal system, and are absorbed; the toxins are carried by the portal vein of the liver to the liver. The liver has many binding sites. The levels of enzymes that metabolize the liver are also high (especially cytochrome P-450) which makes the toxicants less toxic and water-soluble, and hence easier to excrete. However, in some cases, the toxic effect can induce lesions that are centrilobular associated with higher levels of cytochrome P-450.[5-13]

This study aims to explore the acute toxicity test of the ethanol extract of Bidara leaves using white mice as experimental animals. Toxicity observations were carried out by looking at the liver and kidney function of White Rats.

2. Methods

Animal model

A total of 30 white rats (Rattus norvegicus) Wistar strains were obtained from the Eureka Research Laboratory (Palembang, Indonesia) weighing between 200 – and 250 grams. All experimental animals were kept in cages under controlled conditions of 12 hours of the light-dark cycle, temperature 22 ± 1^{o}C and humidity 40-60%, and given ad libitum food. The research treatments and procedures have received approval from the medical research ethics committee of the Universitas Hasanuddin, Faculty of Medicine (No. 123 / kptfkunhas / 2019).

Bidara leaf extraction preparation

Simplicia Bidara leaves were obtained from the Tawangmangu Herbal Research Center, Karanganyar, Indonesia. The process of extracting the leaves of Bidara was carried out by maceration in which 500 grams of simplicia were macerated with 96% ethanol for 72 hours. Next, do the separation between the pulp and the macerate. The macerate was then evaporated with a rotary evaporator (Shimadzu) in order to obtain a thick extract, extract of bidara leaves (EB).

Acute toxicity test

After one week of adaptation, the mice were randomly divided into the following six groups, each containing five animals: Normal control group, EB group (50mg/kg BW), EB group (150 mg/kg BW), EB group (450 mg/kg BW), the EB group (1350 mg/kg BW) and the EB group (4050 mg/kg BW). Bidara leaf extract was given orally for 14 days, where first the extract was made into a suspension by adding a 1% Na-CMC emulgator.

Evacuate blood serum samples

The mice were anesthetized by injecting 10% Chloral Hydrate (3.5 ml/kg) intraperitoneally. Rats were sacrificed by intraperitoneal injection of 10% chlorine hydrate, and then blood serum was taken through the orbital vein. The serum was then centrifuged at 10,000 rpm for 10 minutes, the temperature was 25oC, and the supernatant was stored at -20oC for analysis of SGOT, SGPT, Ureum, and creatinine levels using the spectrophotometer method (Biorad).

Phytochemical test

Bidara leaf extract was analyzed for phytochemical screening which included tannins, alkaloids, flavonoids, quinones, saponins, and steroids/triterpenoids. Bidara leaf extract was separated using TLC as a stationary phase in the form of silica gel GF254 and the mobile phase in the form of n-hexane: chloroform: ethyl acetate (2: 5: 5).

Statistical analysis

All data were presented as mean ± standard deviation, and all statistical analyzes were performed with the SPSS 25 (IBM) program. One-way ANOVA followed by a post hoc analysis was carried out to assess the difference in mean expression levels of each protein. $P < 0.05$ was determined as an indication that there was a significant difference in mean levels.

3. Results and Discussion

This study identified 301 children with ASD, where 97 of them suited the inclusion and exclusion criteria. Those ninety-seven children had dominant male demographic characteristics (87.63%), with age under five years old (73.20%) as shown in table 1.

Table 1 shows the effect of acute toxicity tests on liver function of white rats. This study shows that the extract of Bidara leaves has a relatively high toxic dose, namely at a dose of 4050 mg/kg BW. Bidara leaf extract at doses below 1500 mg/kg BW, shows no toxic effect on the liver.

Table 2 also shows the toxicity test of Bidara leaf extract on the kidney function of white rats. This study shows that the leaf extract of bidara at doses of more than 4000 mg/kg BW has serious toxic potential for the kidneys. Doses less than 1500 mg/kg BW are relatively safe doses for the kidney function of white rats.

Table 1. Level of liver function in serum

No.	Group	SGOT (mg/dL) ± SD	P-Value*	SGPT (mg/dL) ± SD	P-Value*
1.	Control	37.36 ± 9.41	-	36.36 ± 2.41	-
2.	EB 50	35.23 ± 10.43	0.52	36.23 ± 2.43	0.34
3.	EB 150	36.11 ± 11.21	0.45	37.11 ± 1.21	0.21
4.	EB 450	37.12 ± 12.43	0.55	38.12 ± 2.43	0.18
5.	EB 1350	38.11 ± 11.65	0.21	39.11 ± 1.65	0.23
6.	EB 4050	350.83 ± 21.21	0.00	379.83 ± 26.21	0.00

* VS Con; ANOVA. post hoc Bonferroni; $p<0.05$

Table 2. Level of renal function in serum

No.	Group	Ureum (mg/dL) ± SD	P-Value*	Creatinine (mg/dL) ± SD	P-Value*
1.	Control	48.36 ± 9.41	-	1.36 ± 0.41	-
2.	EB 50	47.23 ± 10.43	0.52	1.23 ± 0.43	0.34
3.	EB 150	47.11 ± 11.21	0.45	1.11 ± 0.21	0.21
4.	EB 450	48.12 ± 12.43	0.55	1.12 ± 0.43	0.18
5.	EB 1350	49.11 ± 11.65	0.21	1.11 ± 0.65	0.23
6.	EB 4050	454.83 ± 21.21	0.00	479.83 ± 26.21	0.00

* VS Con; ANOVA, post hoc Bonferroni; $p<0.05$

4. Conclusion

Bidara Leaf Extract has a toxic dose above 4000 mg/kg BW in Wistar White Rats.

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