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Safety Assessment of Combination of *Tinospora crispa* Extract, *Andrographis paniculata*, *Cinnamomum burmanii*, Syzygium polyanthum and *Momordica charantia* in Hepar and Renal Function

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A B S T R A C T

Tinospora crispa, Andrographis paniculata, Cinamomum burmanii, Syzygium polyanthum and *Momordica charantia* 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. Herbal plants, which are rich in phenolic compounds, have the efficacy, among others, as antioxidants, anti-inflammatory, antimicrobial, anti-fungal and prevents tumor growth. 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.

1. Introduction

Tinospora crispa, Andrographis paniculata, Cinnamomum burmanii, Syzygium polyanthum, and *Momordica charantia* 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. Herbal plants, which are rich in phenolic compounds, have efficacy, among others, as antioxidants, anti-inflammatory, antimicrobial, anti-fungal and prevent tumor growth.[1-5] These herbal plants are nutritious for protecting human DNA cells caused by damage from actinic radiation. Although there have been many studies on the chemical content of herbal plants, the clinical use of herbal combinations is still based on empirical evidence so it is still necessary to determine the pharmacological properties and test the toxicity of the extracts.[6-9] 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.[10-14]

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 hydrophilic so that it is easily 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 liver portal vein 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. But in some cases, the toxic effect can induce lesions that are centrilobular in nature associated with higher levels of cytochrome P-450.[15]

This study aims to explore the acute toxicity test of a combination of extracts using white rats 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 200-250 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 treatment and procedures have received approval from the medical research ethics committee of the Faculty of Medicine, Sriwijaya University (No. 123 / kptfkunsri / 2020).

Herbal 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, herbal combination extract (EKH).

Acute toxicity test

After 1 week of adaptation, the mice were randomly divided into the following six groups, each containing 5 animals: Normal control group, EKH group (50mg / kgBW), EKH group (150 mg / kgBW), EKH group (450 mg / kgBW), the EKH group (1350 mg / kgBW) and the EKH group (4050 mg / kgBW). The herbal combination extract was given orally for 14 days, where the extract was first 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, then blood serum was taken through the orbital vein. The serum was then centrifuged at 10.000 rpm for 10 minutes, the temperature was 25^{o}C, and the supernatant was stored at -20^{o}C for analysis of the levels of SGOT, SGPT, Ureum, and creatinine using the spectrophotometer method (Biorad).

Phytochemical test

The herbal combination extract was analyzed for phytochemical screening which included tannins, alkaloids, flavonoids, quinones, saponins, and steroids/triterpenoids. The herbal combination 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

Table 1 shows the effect of acute toxicity tests on liver function of white rats. This study shows that the combination extract has a fairly high toxic dose, namely a dose of 4050 mg/kg BW. The combined extract dosage below 150 mg/kg BW, has shown no toxic effect on the liver.

Table 2 also shows the toxicity test of the combined extract on the kidney function of White Rats. This study shows that Bidara leaf extract doses of more than 4050 mg/kg BW have a serious toxic potential for the kidneys. Doses less than 150 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.	EKH 50	35.23 ± 10.43	0.52	36.23 ± 2.43	0.34
3.	EKH 150	36.11 ± 11.21	0.45	37.11 ± 1.21	0.21
4.	EKH 450	37.12 ± 12.43	0.55	38.12 ± 2.43	0.18
5.	EKH 1350	38.11 ± 11.65	0.21	39.11 ± 1.65	0.23
6.	EKH 4050	350.83 ± 21.21	0.00	379.83 ± 26.21	0.00

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

Table 2. Level of renal function in serum

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

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

4. Conclusion

The combination extract has a toxic dose above 4000 mg / kgBW in Wistar White Rats.

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