Sub chronic diagnosis of administration with Scurrula atropurpurea to blood biochemistry analysis

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Sub chronic diagnosis of administration with *Scurrula atropurpurea* to blood biochemistry analysis

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Abstract. This research is to evaluate whether *Scurrula atropurpurea* has a protective effect on the liver caused by its sub-chronic treatment. The rats consists of four groups: normotensive group and three groups adminsitered methanolic extract of *S. atropurpurea* (MESA) at a dosage of 250; 500; and 1000 mg/kg BW. Serum liver and kidney function were assayed. Serum cholesterol was checked. Histopathology of brain, liver, cardiac, lung and kidney were identified using hematoxylin eosin staining. The administration of MESA250, MESA500 and MESA1000 caused no differences among the groups compared with the control group. Structural evaluations on brain and lungs showed that MESA administration carried no changes. In liver and heart, MESA1000 led to repair tissue damage. But for kidney, MESA250 was safe for the tissue. The results of this study revealed that methanolic extract of *S. atropurpurea* at *antihypertension* dose is safe even when taken for a more extended period. At a higher dose, the extract may have the potential to increase some hematological indices but may induce tissue repair in the liver and heart. The lowest dose is safe for kidney tissue.

1. Introduction

Mistletoe of *Scurrula atropurpurea* (BL.) Dans is hemi parasitic tea plant, especially on the island of Java. The leaves and stems of *Scurrula atropurpurea* (BL.) Dans have been empirically and potential used for therapeutic treatment. Previous studies showed that this plant has function in blood pressure lowering [1,2]. Furthermore, the pathway which this plant can reduce blood pressure is in oxidative stress inhibition and endothelial cell protection.

Scurrula atropurpurea (BL.) Dans is a growing determination that comprehension of conventional medicine is potential not only for its essential as curative herbs but also for its socioeconomic status and cultural ingredients [3]. Although many health issue have been investigated by using medicinal herbs, acute, sub chronic and chronic test of some plants are also reported [4].

Nevertheless, there is no scientific report on the safety of using *Scurrula atropurpurea* (BL.) Dans as a herbal cure. The present study is, consequently, pointed at evaluate the security of *Scurrula atropurpurea* (BL.) Dans by explore chronic toxicity of this herb extract.

Methods

2.1 *Preparation tea parasite crude extract*

Determination and identification the crude extract characteristic of botanical determination was performed at the Indonesian Scientific Institute (LIPI) at Purwodadi, Pasuruan, East Java. "One hundred milligrams of dry leaf powder was steeped in methanol in a 1000 ml-erlenmeyer flask. The mixture was shaken for 30 min to distribute the powder homogenously in methanol. Subsequently, the mixture was left to stand overnight to precipitate. The supernatant, a combination of methanol and the active constituents, was subjected to evaporation. The extract was labeled and stored in a freezer. The methanolic extract of *S. atropurpurea* (MESA) was administered daily by the oral gavage (2 ml) using a catheter for 90 days" [1] [2] [5].

The procedure of experimental animal model were done by the guidelines of Indonesia legislations on the ethical clearance of laboratory animals. The research protocol was approved by the Animal Review Board of the Brawijaya University, Malang, Indonesia. The number of rat which is 40 tail, aged 8-10 weeks and weighing 180-200 g. The treatment groups were divided the normotensive group, the MESA treatment at dosages of 250, 500, and 1000 mg/kgBW. The rats were assigned randomly into the groups; each group contained ten rats. After 90 days treatment, the blood and tissue were determined [5].

2.2 *Analysis of liver function and lipid profiles*

After sacrifice, blood samples were collected by left ventricular blood. Liver function of animal model was recognized based on changes in plasma levels of glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT). Plasma levels of albumin, total protein, alkaline phosphatase (ALP), total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL- C), high-density lipoprotein cholesterol (HDL-C) were measured using Spotchem EZ SP 4430 (ARKRAY Inc, Kyoto, Japan).

2.3 *Histological examination*

The brain, liver, heart, and kidney were placed in 25 mM KCl & Phosphate-buffered saline (PBS) to relaxing buffer, then will keep in neutral-buffered formaldehyde 40% at room temperature. Hematoxylin and eosin (H&E) stained sections (~5 µm) were prepared to measure histopathology [6]. The sections were photographed at 400x magnification using an Olympus (Tokyo, Japan). Microscope lighting, focus, and field selection were optimized for distinction of cell boundaries. Images were opened in Image J and after setting the threshold, analyzed. Data from all the fields were combined and then analysed [].

2.4 *Data Analysis*

Data are performed as mean ± SEM. The research data analysed by one-way analysis of variance (ANOVA), and continued with a post hoc analysis was evaluated using Fisher's least significant difference (LSD) test. A *P*-value of less than 0.05 was considered to be statistically significant.

Tabel 1. Effect of subchronic administration of MESA in blood serum levels of rats.

Group	Protein	Albumin	Globulin	SGOT	SGPT	Cholesterol	Triglycerida	BUN	Creatinine	Bilirubin	Urea
C	7.34	3.84	3.51	239.67	147.67	75.00	66.00	22.50	0.45	0.02	48.10
G1	7.81	3.44	4.37	198.56	90.33	51.67	44.78	24.40	0.54	0.07	51.69
G2	8.37	3.38	4.93	216.50	88.00	59.83	63.67	26.52	0.62	0.03	56.88
G3	8.59	3.64	4.93	210.22	104.00	54.00	43.78	24.20	0.58	0.03	51.78

Results

3.1 Effect of subchronic administration of MESA on blood serum measurement

Oral administrations of MESA at 250, 500, and 1000 mg/kg BW did not causing any mortality in rats during the observation period. Furthermore, there were no unconcealed signs and symptoms indication of toxicity in study groups. Base on in table 1, subchronic administration of MESA in all groups did not significantly influence of the examined blood serum indicator to the control. Previous studies revealed that administration of MESA caused no toxic effect in the biochemical indices [5];[7];[8];[9];[10]. Figure 1 (a-k) reveals that MESA did not cause dysfunction in liver and kidney function. Furthermore, lipid profile was normal in comparison with the control.,Cholesterol profiles are found no differences in female rats with subchronic administration of MESA [11]. Furthermore, treatment with MESA subchronically did not affect cell necrosis in the liver because MESA contains many active substances such as quercetin that inhibit tissue damage-induced by free radical [12].

3.2 Effect of subchronic administration of MESA to wet organ weights

Necropsy macroscopic observation of the research studied visceral organs, such as brain, pulmo, cor, hepar and kidneys for any feasible changes in position, size, shape, and color did not disclose any obvious abnormality. Wet weight (in g) and respective organ weight (in g per 100 g body weight) of brain, hepar, lung, heart, and kidney of both extract-treated and normotensive groups. No significant difference was investigated in absolute and relative organ weights of MESA and normotensive groups [6]. No significant differences between absolute and relative organ weights of the kidneys were investigated in normotensive and MESA.

3.3 Effect of subchronic administration of MESA on histopathology of brain and lung

Histological examinations of liver sections of rats treated with 250, 500, and 1000 mg/kg b.w MESA showed a normal architecture of brain and lung in comparison with control group.

3.4 Effect of subchronic administration of MESA on histopathology of the hepar

Histological observation of hepar frontal sections of rats used with 250 and 500 mg/kg b.w MESA appeared a normal features with normal appearance of the central vein and hepatic sinusoids lined with endothelial and *Kupffer* cells similar to the normotensive groups. The hepatocytes showed normal in shape and size, and no available of vacuoles were prominent in cytoplasm. In the rats group used with 1000 mg/kg b.w MESA, however, there was cellular damage of hepatocytes. Furthermore, central veins and portal triads were infiltrated and enlarge by mononuclear leukocytic cells, indicating mild hepatitis [6].

3.5 Effect of subchronic administration of MESA on histopathology of the heart

Histological observations of hepar frontal sections of rats treated with 500 and 1000 mg/kg b.w MESA appeared a normal features with normal appearance of the cardiomyocytes compared to the control group. There was a few cardiomyocytes damage showed in rats administered with 250 mg/kg b.w MESA.

3.6 Effect of subchronic administration of MESA on histopathology of the kidney

Histological study of kidney frontal sections of rats administered with 250 mg/kg b.w MESA revealed a normal features with normal appearance of the nephrons compared to the control group. Moreover, this normal architecture were found both in right and left kidney. There was damage in glomerular structure showed in rats treated with 500 and 1000 mg/kg b.w MESA. These mean that subchronic administration of MESA should be less than 500 mg/kg b.w MESA to protect structural damage of kidney.

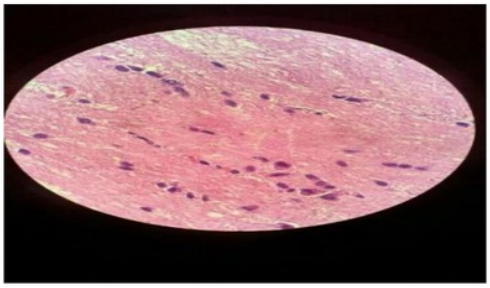

Figure 1. Histopathology of the brain after administration of MESA (Olympus CX21, 40x10).

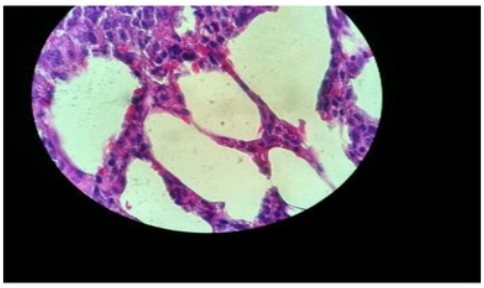

Figure 2. Histopathology of the lung after administration of MESA (Olympus CX21, 40x10).

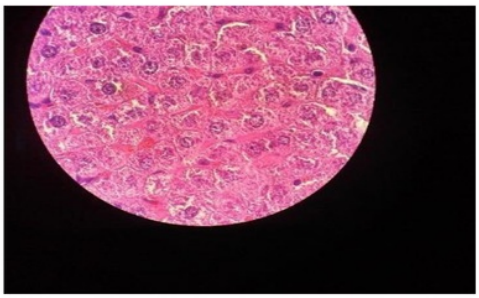

Figure 3. Histopathology of heart after administration of MESA (Olympus CX21, 40x10).

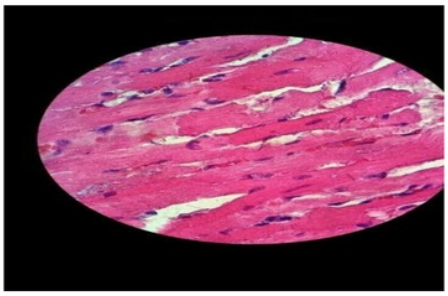

Figure 4. Histopathology of the kidney after administration of MESA (Olympus CX21, 40x10).

Discussion

Study in female rat with subchronic administration of MESA cause no tissue damage in the heart [13], lung [14]. Therefore, subchronic experiment in this research also showed that the MESA was well tolerated by all animal model, as there were mortality and MESA-correlated marker and symptoms of toxicity test were examined all round the study phase. The MESA had non toxic or damaging effect on body growth marking of all treatment groups. The function of whole blood as indicator of physiological and pathological category in animals and humans is well recorded [6] [15]. In acute study test and sub chronic test, changes in biochemistry analysis in addition biochemical indicators are normally treat as lead of harmful or toxicities test. In the current study, MESA treatment of 250, 500 or 1000 mg/kg b.w of MESA for 90 days did not appear the standar of blood serum indicator. The non clinical security study guidance for herb products that have better cause for treat or calculated for a long time of use. In accordance with these principles, levels of serum total protein, ALP, ALT, and AST were measured in the present study and used as liver function tests, while creatinine and urea standar were used as kidney function study [16].

The biological characteristics; correlation bertween body weight and organ weight is that in the major part of study, except for the brain. Besides, all of organ are commonly observed to decide whether the measurements of organ has changed, especially concerning the weight of the all animal as indicator. It is for this reason that this study investigated the effect of sub chronic experiment of MESA on absolute and relative organ weights. Histopathological indicator of brain, lung, heart, liver, and kidneys. The current study, no scientific confirmation has ever specify the effects of MESA on organ weights of rat [6].

The major target organ is hepar. Toxicity of the hepar organ, damage to the hepar cause affect of hepatocytes causing to release the membrane-bound enzymes (e.g. ALT and AST), injury to hepato-biliary system thereby leading to release the essential enzymes (e.g. ALP), and/or harmful the catabolic capacity and biosynthetic of the liver function. Thereby appear histopathological damage [17].

The concentration level of the kidney function measured such as serum of urea creatinine, and BUN levels were significantly different in the normotensive and MESA group. Other than, histological investigation contrast of the kidney frontal sections of the investigated rat and normotensive rat. There is no significant differences to MESA experiment. In all treatment groups showed that the tubular renal and glomerular were intact and normal feature . Consequenty, not found a glomerulonephritis [18] after exposure with the MESA. Thus MESA were not cause toxic in the kidney function.

Therefore, this study suggests that the subchronic administration of *S. atropurpuera* is safe for adjuvant with treatment of herbal medicine. On further research, there needs to be a clinical trial in humans. However, nonappearance of any mortality in animal model. The long term, MESA as a remedy antihypertensive herbs

Conclusion

The results of this study indicated that the methanolic extract of *S. atropurpurea* at antihypertension dose is safe for a long term period. In high doses, MESA will trigger damage to organ tissue and increase levels of blood biochemistry. Other hand the lowest dose is safe for organ tissue.

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