**AENSI Journals**

**Advances in Environmental Biology**

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/

# Evaluation of Methanolic Extract of *Scurrula Atropurpurea* (Bl.) Dans Sub-Chronic Exposure On Wistar Rat Liver

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**ARTICLE INFO**

***Article history:***
*Received 28 September 2015*
*Accepted 15 November 2015*
*Available online 24 November 2015*

***Keywords:***
*liver protection, Scurrula atropurpurea (Bl.) Dans*

**ABSTRACT**

**Background:** This study aimed to analyze sub-chronically exposure of methanolic extract of *Scurrula atropurpurea* (Bl.) Dans (MESA) on rat liver function such as AST (aspartate transferase), ALT (alanine transaminase), level of serum albumin, level of serum globuline level of total serum protein, and histopathology of liver. **Objectives:** Male Wistar rats were randomly divided into four groups: control, MESA I (250 mg/BW), MESA II (500 mg/BW), MESA III (1000 mg/BW). Methanolic extract was given once daily per oral for 28 days. Body weight were measured twice weekly. Data were analyzed by one way ANOVA test and continued by *Post Hoc* test on SPSS 21 software. **Results:** No effect were found on orally exposure of MESA for 28 days compared to the control, on rat level of serum AST, serum ALT, level of serum albumin, level of serum globuline and level of total serum protein ($p > 0.05$). That besides no abnormality in liver histopathology in treatment. **Conclusion**: This study concluded sub-chronic exposure of MESA did not interfered rat liver function. It would be a recommendation for next clinical study



**To Cite This Article:** Nour Athiroh, Erna Sulistyowati, Evaluation Of Methanolic Extract Of *Scurrula Atropurpurea* (Bl.) Dans Sub-Chronic Exposure On Wistar Rat Liver. ***Adv. Environ. Biol., 9(23),*** *245-250, 2015*

## INTRODUCTION

*Scurrula atropurpurea* (Bl.) Dans is a a half parasitic plant cause has chlorophyll and remains able to assimilate itself, suck the water as well as organic and inorganic tea from host plant. Botanical classification of *Scurrula atropurpurea* (Bl.) Dans is class Dicotyledonae, Santales order, Loranthaceae family. Scurrula has several species; *Scurrula (S) oortiana, S. atropurpurea, S. junghuni, and S.parastica*. Scurrula *atropurpurea* extract contains 16 bioactive materials comprising of six fatty acid compounds, two santin, two monoterpenes glycosides of the flavonol glycosides, one lignan glycosides, and four flavones [1,2]. Several types of group of Loranthaceae family potentially have antihypertensive. Many studies reported possibility of parasitic plant on tea crop had antihypertensive role, one is *Viscum album.* Administration of *Viscum album* for 3-5 weeks abled to reduce blood pressure on hypertensive patient [3,2].

Other types of parasitic plant on tea, *Scurulla oortiana* and parasitic on guava roses (*Macrosolen javanus*) had capability to decrease contraction of separated arterie on rat in vitro study [2]. Previous study, *S. atropurpurea* showed its ability on lowering blood pressure in DOCA-salt hypertensive rats, through improvement of endothelial dysfunction and oxidative stress [4,5,6]. The activity of active ingredients in *S. atropurpurea* are antiantioxidant which inhibit oxidative damage caused by free radicals.

The leaves and stems of these plants contain alkaloids, flavonoids, glycosides, triterpenes, saponins, and tannins that act as antioxidants. Potential flavonoids as antioxidants can reduce the activity hydroxy radical, superoxide anion and peroxide radicals fat [1,2].

Based on these studies, we evaluate sub-chronical administration of *S. atropurpurea* on liver function to show its safety. This study generated to standardize it as an antihypertensive medicinal preparation. For that reason we aimed to analyse level of liver enzymes; ALT, AST, albumin, globulin, and total protein in Wistar rats that were given *S. atropurpurea* for 28 days.

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*Methodology:*

*Preparation of tea parasite crude extract:*

Preparation of tea parasite crude extract *Scurrula atropurpurea* was determined biologically at the Laboratorium of Material Medica Batu East Java. MESA was obtained through several steps. The leaves were washed, dried in an oven at 40-60$^0$C, then ground into a powder. A 100 mg portion of this powder was steeped in methanol in a 1 L Erlenmeyer flask. The mixture was shaken for 30 minutes to distribute the powder homogenously in the methanol. To collect the precipitate, the mixture was left to stand overnight. The upper layer know and supernatant, being a mixture of methanol and the active constituents, was subjected to evaporation. The extract was then labeled [4,5,6,7].

*Animal studies:*

Male Wistar rats, weight 200-300 gram, were acclimatized for 7 days, grouped randomly, and put on cages according to weight evenly (weight variation less than 20% of average weight. The rats were fasted for 14-18 hours before it had given treatment. Drinking water were provided. After fasted, Rats were weighed and given MESA by oral gavage. Once treated, the feed should be given back after 3-4 hours. The dosage of MESA defined on 500 mg/kg/BW as previously described [4,5,6,8]. MESA administered daily for 28 days with volume of dilution was 1 ml/100g BW. Body weight measured twice a week. At day 29$^{th}$ rats were sacrified and blood was drawn to observe levels of AST, ALT, albumin, globulin and total protein serum level.

*Blood Samples:*

The blood samples were collected, left to clot in clean, dry tubes, and centrifuged at 3000 rpm (600 g) for 10 minutes using Heraeus Labofuge 400R, Kendro Laboratory Products GmbH, Germany, to separate sera. The sera were kept in a deep freezer at −20°C until biochemical markers were analyzed within one week.

*Liver Biomarkers:*

All biochemical measurements were determined in serum according to the details given in the kit's instructions and performed by using a Shimadzu UV-VIS Recording 2401 PC (Japan). The activities of cellular enzymes such as AST and ALT were determined according to the methods of Reitman and Frankel [(9)], while the concentrations of albumin, globulin and total protein were determined according to the methods of Lowry *et al*., [10, 11, 12, 13, 14] respectively.

*Histopathology of Liver Tissues:*

Adequate fixation is crucial to the success of histopathological evaluation. Approximately twenty times the volume of 10% neutral buffered formalin (NBF) relative to the amount of tissue to be fixed should be used. Tissue samples should be less than 5mm thick to ensure thorough fixation. After tissue specimens are fixed in 10% neutral buffered formalin, they are dehydrated in graded alcohols, embedded in paraffin, sectioned at 3-5 µm, stained with hematoxylin and eosin (H&E), and cover-slipped for standard light microscopic histopathological interpretation by the pathologist[15]. Histological slides were photographed under Olympus photomicroscope.

*Statistical analysis:*

Statistical analysis was done using SPSS 17 for windows and the values were expressed as mean ± S.D. The statistical significance of differences between the means was analyzed using one-way analysis of variance (ANOVA) followed by Duncan's test for comparison between different treatment groups. Statistical significance was set at $p<0.05$.

*Ethics:*

This study had certified by Ethic commission of Faculty of Medicine, University of Brawijaya, Indonesia with letter approval number: 369/ EC/ KEPK/06/2015.

## RESULTS AND DISCUSSION

*Signs of Toxicity:*

No mortality occurred during the study period. We observed no symptoms and no signs of toxicity such as hyperirritability on rats. In addition, there was no effect on food and water consumptions in all groups (in tabulated data).

*Liver Biomarkers:*

The results demonstrating relatively normal on level of liver biomarkers. There were unsignificant increases in AST and ALT in all groups (Table 1). AST serum level raised in each group (Figure 1) and also level of ALT

(Figure 1). However, albumin, globulin and total protein levels were normal in all rats (Figure 1). Supplementation of MESA 250, 500 and 1000 mg/kgBW daily for 28 days showed no changes of compare to the control groups.

**Table 1:** Liver function biomarkers and liver weight in treatment group

| Group | Liver function biomarkers (U/l) X̄ ± SD | | | | | |
|---|---|---|---|---|---|---|
| | AST | ALT | Albumin | Globulin | Total Protein | Liver Weight (gr) |
| Control | 124.80 ± 19.33 | 74.40 ± 13.93 | 3.35 ± 0.22 | 3.45 ± 0.43 | 6.80 ± 0.38 | 7.64 ± 1.23 |
| MESA I | 136.20 ± 22.60 | 77.80 ± 19.09 | 3.35 ± 0.11 | 3.56 ± 0.60 | 6.91 ± 0.53 | 6.64 ± 0.85 |
| MESA II | 149.00 ± 24.60 | 65.40 ± 11.01 | 3.40 ± 0.19 | 3.42 ± 0.41 | 6.82 ± 0.53 | 6.38 ± 0.79 |
| MESA III | 122.80 ± 7.46 | 67.60 ± 16.71 | 3.55 ± 0.13 | 3.21 ± 0.35 | 6.76 ± 0.25 | 7 ± 1.02 |

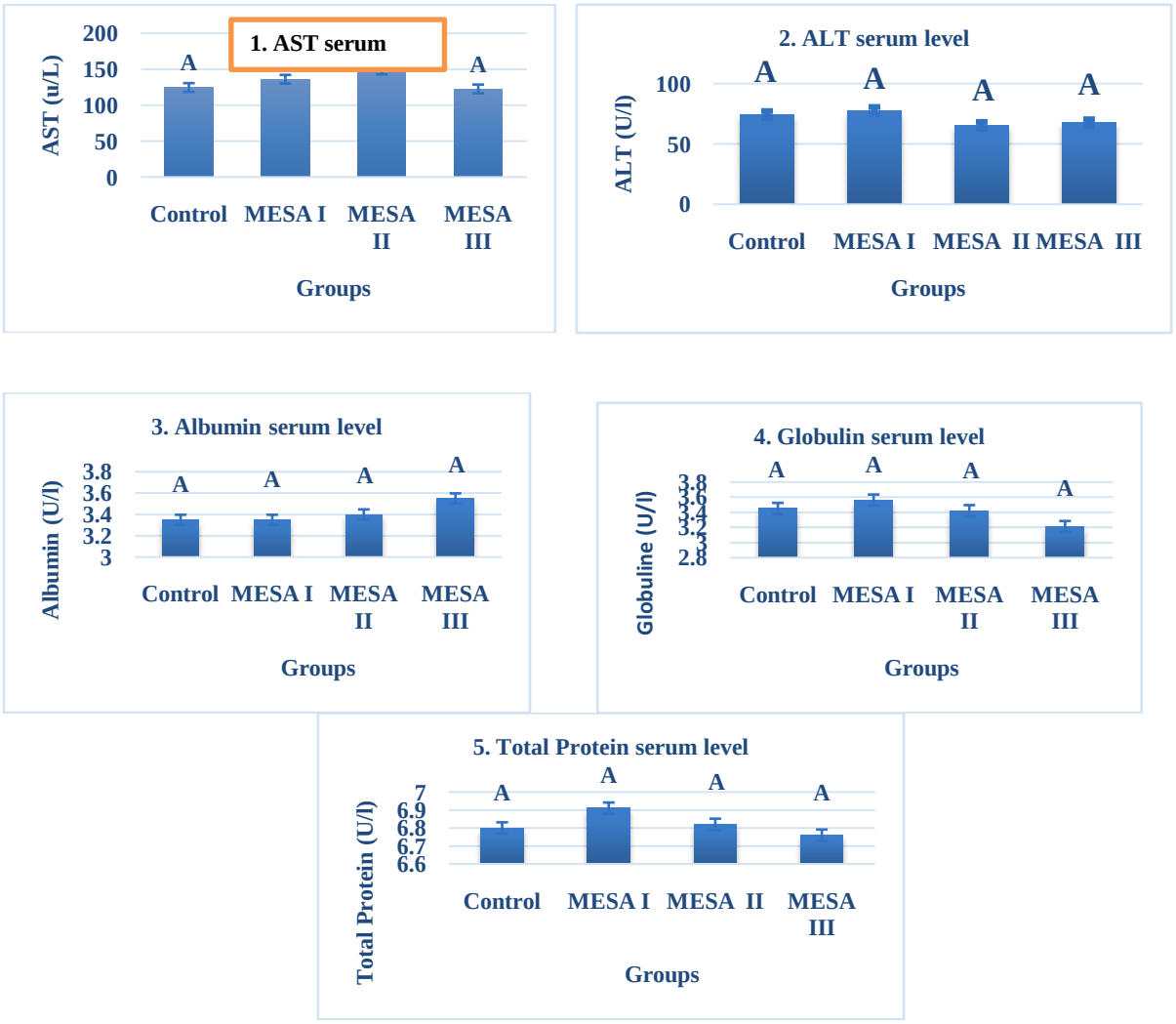


**Fig. 1:** Serum exposed to MESA for 28 days. The values represented are the means ± S.D. A = means having the same letters are not significantly different from each other, $P \geq 0.05$. Groups: control, MESA 1 = 250 mg/kg BW. MESA 2 = 500 mg/kg BW and MESA 3 = 1000 mg/kg BW

*Histopathology of Liver:*

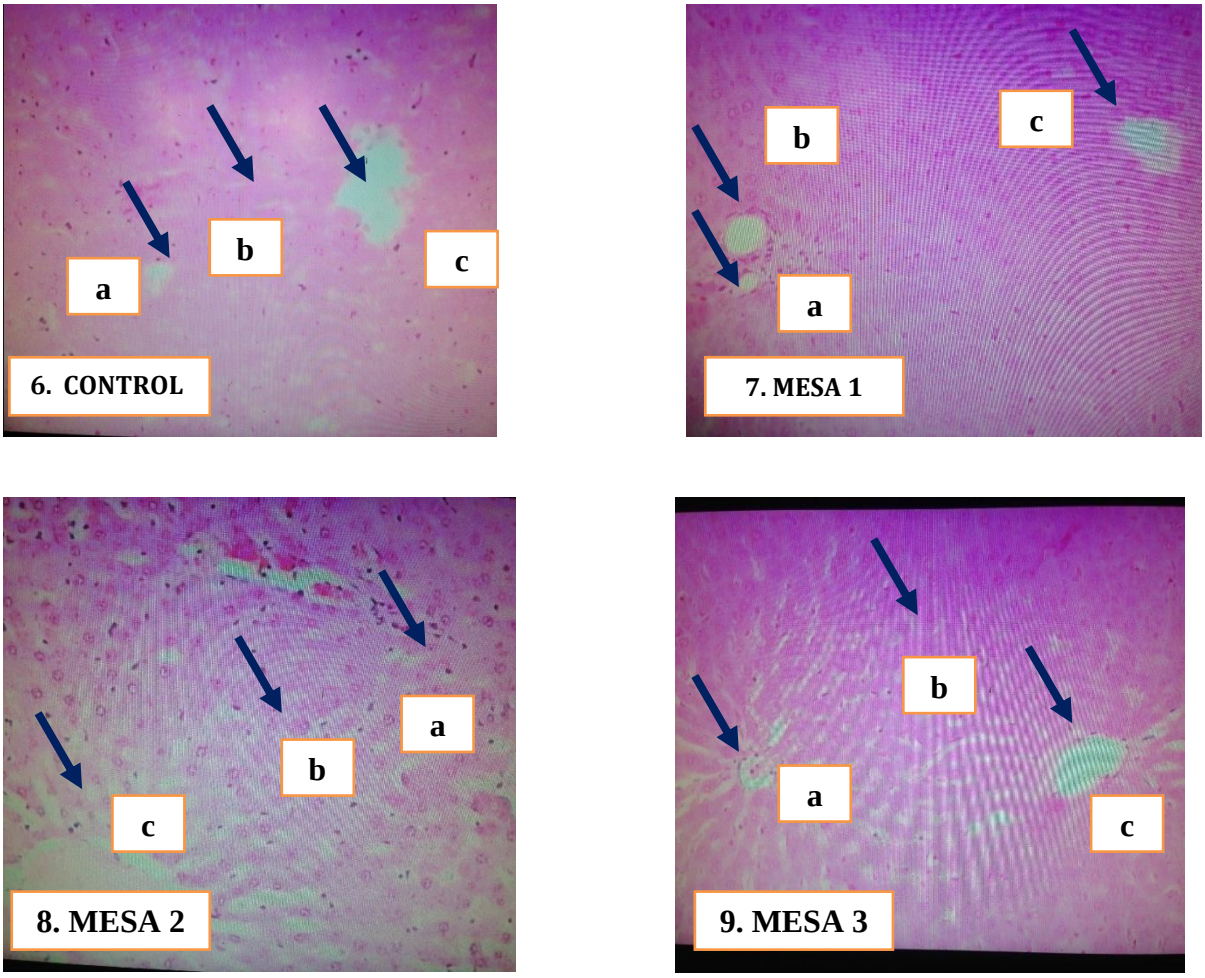


**Fig. 2** Histopathology of rat liver exposed to MESA for 28 days. There is no abnormality effect. H.E. X 100. a = A portal triad ; b = vena centralis ; c = nucleus cell

Results of the present study demonstrate that subchronic administration of MESA did not produce signify toxicity. Providing MESA cause significant increase in body weight gain in rats. There is no effect on feed and water intake in treated rats. In addition, there are no differences in MESA groups compared to control on liver function. Other study, Amin, *et. al* showed ethanolic extract of *Phyllanthus niruri* and *Melastoma malabathricum* able to improve liver function due to increasing immune system[16].

Liver function biomarkers are a helpful screening tool, which are an effective modality to detect hepatic dysfunction. Our results showed that MESA have no caused in liver enzymes, that is, AST and ALT in Wistar rats. In fact, aminotransferases are intracellular enzymes, most frequently utilized, and specific indicators of hepatocellular necrosis. AST and ALT catalyze the transfer of the amino acids of aspartate and alanine, respectively, to the keto group of ketoglutaric acid.

In the present study, oral administration of MESA to Wistar rats disable cause changes in serum total protein, albumin and globulin levels. This study proved MESA has no effects in causing liver dysfunctions and disturbance in the biosynthesis of protein. Several tea parasite noted that several studies showed Loranthaceae family in various feeding experiments have no toxicity. those tests carried out suggest that the crude mistletoe leaf extracts of Loranthaceae family were non-toxic to laboratory animals [17].

Other potential role of MESA is protecting from microbes. It competent to kill bacteria. *Scurrula atropurpurea* inhibit 50% concentration of *Enterobacter sakazakii* effectively [18]. Other studies tested it suppressed *Bacillus subtulis, Klebsella pneumaneae, Vibrio cholerae*, and *Esherichia coli*. *Scurrula atropurpurea, Macrosolon cochichinensis* and *Viscum album* are hemiparasites found in the forests of South West Bengal, showed antimicrobial activities against four bacterial strains *(Bacillus subtilis, Klebsella pneumoniae, Vibrio cholerae* and *Escherichia coli*). With regard to their natural distribution in the forests of southern parts of west Bengal it was observed that the *Scurrula atropurpurea* and *Viscum album* are now very scant and difficult to locate many times in the field [19]. Other hand that some chemical entities transferred from host *Dendrophthoe falcata* and *Mangifera indica* to the parasite *Scurrula parasiica* are responsible for its effect on blood sugar level [20].

The figure 2 showed that histopathology of liver in rat administered with MESA sub chronic 28 day. The result, no e No abnormality was found in the histopathology of the liver, kidney, heart, and lung in the experimental group of rats following same dose of two compounds isolated from *Loranthus globosus* Roxb when compared with control group. This preliminary study suggest that isolated compounds may used safely for clinical trial [21].

*Conclusion:*

The result, no e No abnormality was found in the histopathology of the liver, kidney, heart, and lung in the experimental group of rats following same dose of two compounds isolated from *Loranthus globosus* Roxb when compared with control group. This preliminary study suggest that isolated compounds may used safely for clinical trial.

*Conflicts of Interest:*

The authors declare that there are no conflicts of interests regarding the publication of this article.

## ACKNOWLEDGMENT

The authors thank to Direktorat Jenderal Pendidikan Tinggi, Kementerian Pendidikan dan Kebudayaan, for funding on penelitian Strategis Nasional letter number : 018/SP2H/P/K7/KM/2015, on April 2nd 2015.

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