Research Article

# Proteomic Profile of Blood Plasma of *Mus Musculus* in the Acute Toxicity Test of Single Black Garlic (*Allium Sativum*)

**Lia Siti Halimah[1*], Khomaini Hasan[2], Dewi Ratih Handayani[2], Nurjannah Achmad[2], Rini Roslaeni[3], Dwi Sulistyowati[4], and Regita Zalza Zulkarnaen[4]**

[1]Research and Publication Center, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia
[2]Department of Biochemical, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia
[3]Department of Clinical Pathology Anatomy, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia
[4]Medical Study Program, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia

**ORCID**
Lia Siti Halimah: https://orcid.org/0009-0008-1176-5574

Corresponding Author: Lia Siti Halimah; email: lia.dio28@gmail.com

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**Abstract.**

This study aims to obtain overall information about all proteins formed in body fluids, cells, or tissues at a certain time and to analyze the mechanism of cell responses to various types of stress and drugs. Single black garlic (SBG) has the potential to be a pharmacological agent. However, the safety of using single black garlic is still unknown, so a material toxicity test is needed. This research is a laboratory experimental research with a post-test-only control group design. An oral acute toxicity test was used. Observation time was 14 days and consisted of four treatments, negative control, SBG dose 2000 mg/kgbw (P1), 3000 mg/kgbw (P2), and 5000 mg/kgbw (P3). Post-treatment protein analysis was done using the SDS-PAGE electrophoresis method. Observations of clinical symptoms of SBG at doses of 2000 mg/kgbw, 3000 mg/kgbw, and 5000 mg/kgbw showed no clinical symptoms in mice, both motor activity and pupils were still normal, no convulsions (seizures), lacrimation, paralysis or death were seen in mice. Observations on plasma proteins showed differences in protein between the treatments at the SBG dose of 2000 mg/kgbw with the SBG dose of 3000 mg/kgbw and 5000 mg/kgbw, but the four treatments have the same type of protein in the protein band with a molecular weight of 25 kDa. So, it can be concluded that SBG doses of 2000 mg/kgbw, 3000 mg/kgbw, and 5000 mg/kgbw are not toxic to mice but can stimulate the expression of certain proteins.

**Keywords:** Single Black garlic (SBG), oral acute toxicity test, proteomic analysis, SDS-PAGE

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## 1. Introduction

Proteomic analysis is a method to separate, identify and quantification of the entire complement proteins expressed by the genome, cell, or tissue. 1 This analysis is useful to get all the information of all proteins formed in body fluids, cells or tissues certain time as well as to analyse cell response mechanisms to various types of stress and drugs [1].

Majority proteomic analysis use plasma proteins blood to determine activity, distribution, rate of excretion or metabolism, and the toxicity of many pharmaceutical agents in body, where this is due to existence albumin protein which acts as receptors. 2 In addition, the use of the method protein separation in the form of Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS PAGE) be most protein separation method widely used in analysis proteomics due to its reproducibility good at determining molecular weight protein. 3 The pharmaceutical agent in focus The mainstay of medical research is plants herbs, such as single black garlic [2].

Single black garlic is Garlic fermented product (Allium sativum) obtained through Maillard reaction in the form of heating at high temperature with a temperature range between 60-90°C and 70-90% humidity during 10-14 days. During the fermentation process, compounds in garlic which causes a sense of smell the sting will be changed naturally into a stable and safe compound, which will then produce a garlic with sweet taste and sour, more tender and has chewy texture like jelly [3,4].

Recent studies show that single black garlic (SBG) and compounds It has various bioactive activities more biological and pharmacological properties either among other things able to improve antibacterial activity so it can prevent bacterial infection, able to improve blood circulation in patients heart disease through increase antioxidant activity, enhance the activity of the antioxidant compounds able to inhibit the accumulation of compounds Advanced glycation end products (AGEP) which causes diabetes. as well as able Protects the liver by reducing levels Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), and Lactate Dehydrogenase (LDH) in the blood [3].

Safe use of SBG as medicine or seasoning cooking is still little known, so there is an important reason to test the toxicity of this single black garlic. Test Acute toxicity was used to test toxicity in experimental animals within 24 hours. Symptoms of acute toxicity of an pharmaceutical ingredients, can be known based on clinical symptoms that occur

over time certain time after induction, such as effect tremors, lacrimation, paralysis, and diarrhea, based on changes in body weight as well histopathological picture [5].

Based on research by Marie and Wijayanti who used the extract black garlic from 0-2000 µg/mL indicates that garlicfresh and single black onion (SBG) did not show toxicity to cells Vero at all concentrations. However, research conducted by Permatasari et al stated that otherwise single single black garlic potentially decrease cell viability and able induces apoptosis of T47D cells despite the highest concentration range reach more than 2000 g/ml Study another conducted by Rumaseuw et al indicates that the provision of onions single black up to a dose of 2000 mg/kgbw does not cause toxic symptoms and there is no change in the behavior of the test animals [6].

Based on the data above, researchers are interested in doing the test SBG acute toxicity using the toxicity test method acute oral and proteomic analysis with using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE).

## 2. Methods

The research design is experimental laboratory with Post Test Only with Control Group. In this research focused on testing for single acute toxicity of black garlic (Allium sativum) and proteomic profile analysis against Mus musculus.

The Twenty four of Mice were divided into 4 groups given the following treatment Group 1 (KN): as a group negative control, not given any treatment except water and pellets. Group 2 (P1): given the extract black garlic dose of 2000 mg/kgbw. Group 3 (P2): given the extract black garlic dose of 3000 mg/kgbw. Group 4 (P3): given the extract black garlic dose of 5000 mg/kgbw. Mice were fasted before administration of SBG extract for 3-4 hours. Maximum volume administration of the test is 1 ml for mice. After 30 minutes post induction observed for clinical signs resulting intoxication motor activity, state of pupils, convulsions (seizures), lacrimation, paralysis, and death. Then observed periodically every 4 hours for 24 hours up to the 14th day in each group of animals [5].

On the 14th day, carried out blood sampling. The blood obtained is in the form of centrifuged blood plasma beforehand, then the blood plasma is tested protein profile. The proteomic profile is determined by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis method (SDS PAGE), through several stages namely protein sample

preparation, gel preparation polyacrylamide, chamber assembly and glass plate, protein sample induction, process running Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE), and the coloring process as well polyacrylamide gel wash [2].

Data analysis was carried out by descriptive in the form of a table of results observation of clinical signs of intoxication such as motor activity, pupillary state, convulsions (seizures), lacrimation, paralysis, and death and protein molecular weight of induced mouse blood plasma SBG through analysis SDS-PAGE.

This research got approval ethics committee from the Ethics Committee of the Faculty of Medicine General Achmad Yani University with ethics number 019/UH1.10/2022.

## 3. Results and discussions

SBG acute toxicity test single dose, performed several types Observations include 6 categories of symptoms clinical, including motor activity, state pupils, convulsions (seizures), lacrimation, paralysis, and death, were observed in the first 30 minutes post induction to 14 days post induction.

Mice Motor Activity 30 minutes until the 14th day after Onion Induction Black Single indicates that overall onion extract treatment single black given orally in mice do not cause symptoms intoxication on the motor activity of mice. This is proven through movement active mice after 30 minutes post induction to day 14 post induction without discovering behavior animals such as walking backwards and walking use stomach.

This also applies to parameters other clinical symptoms, where at 30 minutes until the 14th day after SBG induction, the condition of the pupils of mice still normal size without occurrence narrowing or widening pupil, mice that are still active without the occurrence of body movements that do not controlled (convulsions), the eyes of mice that still clear without the occurrence of irritation or red eyes accompanied by watery discharge eyes (lacrimation), can still move the extremities (not experiencing paralysis) and not found dead mice.

Based on the observation of symptoms clinical post-induction SBG prove that there is none active compound in it which can cause damage to nervous system and digestive system of mice. The results of this study prove statement by Sembiring and Iskandar which states that black onions alone has a beneficial effect on memory and the nervous system through effects anti amnesic, increased interference cognitive function, and

prevention of inflammation nerves and neurotoxicity and not have a toxic effect on consumer [7].

In addition, the research by Rumaseuw et al also stated that onion extract single black does not cause symptoms toxic and no change in behavior of animals is good from observation central nervous system and nervous system autonomous [8].

Blood plasma protein profile overview Oral induction of onion extract mice single black with various concentrations can be seen by the blue bands formed from an SDS-PAGE check. An SDS-PAGE check is performed using plasma samples mice taken on day 14 post induction of SBG extract. SDS PAGE results are displayed on Figures 1 and 2.

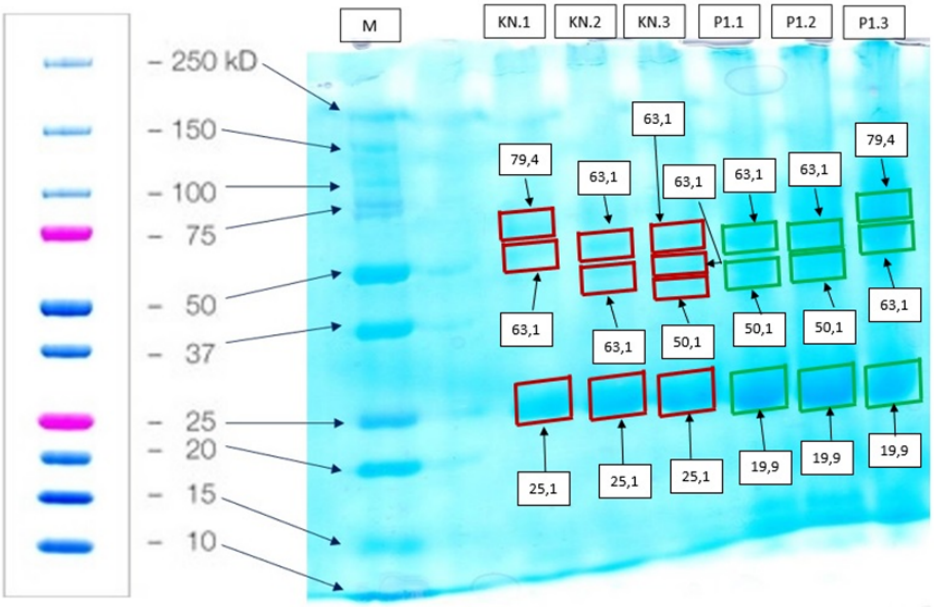


**Figure** 1: Protein bands of single black garlic extract. Description: KN : Negative Control Group, P1 : Black Garlic Extract Induction treatment Group Single dose of 2000 mg/kgbw.

Based on Figures 1 and 2 shows that the majority of the picture the pattern of protein bands almost have the same that is, it has a pattern of parallel thick bands with the 7th marker tape by weight 25 kDa molecule. According to Cahyarini et al. in Nugraha et al, the difference between thick and due to the thinness of the band formed by different numbers of migrated molecules, thick bands is the fixation of several bands.

Ribbons that have ionic strength larger ones will migrate further than bands of low ionic strength. In addition, there are other factors that differentiate the intensity of the band thickness proteins including: (1) there is a difference genetically between these proteins; (2) There are differences in protein concentrations in blood plasma; and (3)

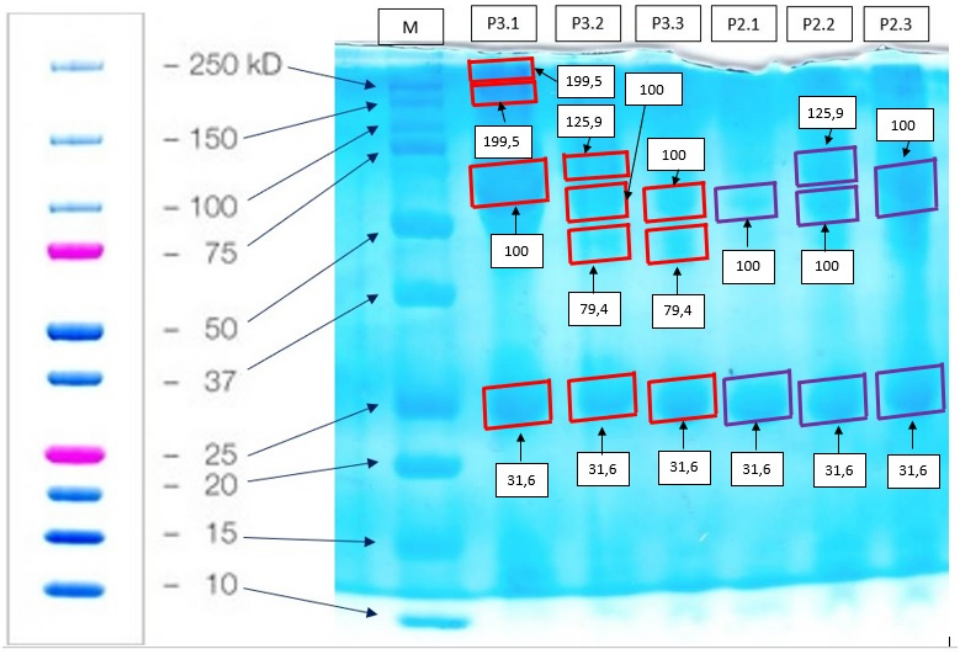


**Figure** 2: Protein bands of single black garlic extract. Description: P2 : Black Garlic Extract Induction Treatment Group Single dose of 3000 mg/kgbw, P3:Single Black Garlic Extract Induction Treatment Group dose of 5000 mg/kgbw, M : marker (standard protein).

the thinness of the ribbon due to less optimal process separation of proteins so that they have bands with a BM of less than 20 kDa.

According to Roy and Kumar, appearance and thickness of protein bands depending on the molecular weight of the protein acrylamide concentration used to make a gel, a voltage is applied on the gel and the length of time the voltage is applied [9].

In addition, Figures 1 and 2 as well shows that the whole treatment have protein bands parallel to 25 kDa BM marker tape. This matter prove that the protein is 25 kDa it remains in the blood plasma regardless undergo induced changes single black garlic extract. Protein identification results with The molecular weight of 25 kDa is estimated to belong to globulin proteins. Globulins work as a carrier of fats, vitamins, hormones and minerals. Blood plasma contains gamma globulin, i.e. immunoglobulin plays a role in immune system. Gamma-globulin function as an antibody. Globulins used to form fibrinogen musculin, crystallin, and antibodies. In addition, it is also estimated at negative control treatment and treatment 1 albumin was found in plasma blood with a molecular weight of 63.1. Albumin is one of the main proteins in the blood which has many functions is also a part important in the structure of blood plasma. Albumin functions include guarding fluid balance in the body, as intercellular bond forming agent its presence is required in the process cell regeneration and repair, binding all parts

of the cell with water so form blood fluids as well as transport various nutrients and hormones. Blood plasma besides containing the proteins albumin and globulin are also present other proteins such as fibrinogen, glycoproteins, haptoglobulins, and 10 lipoprotein, which was not identified in SDS-PAGE [10].

That treatment P1 differ in one type of protein with the KN group namely emergence a new protein band with a BM of 19.9 kDa. In addition, P1 has the same protein with the KN treatment of 63.1 kDa, 79.4 kDa and 50.1 kDa. Treatment P2 and P3 has the same protein with an average molecular weight obtained, namely 31.6 kDa, 100 kDa and 125 kDa. However, on P3 treatment had protein that did not owned by other treatments viz protein with a molecular weight of 199.5 kDa. From these results can be drawn the conclusion that when mice induced with SBG extract Black Single 2000 mg/kgbw, average The protein in plasma is still the same with the control group (KN), but additional protein expression occurs has a BM of 19.9 kDa. However, when mice induced with the extract Single Black Garlic 3000 mg/kgbw and 5000 mg/kgbw, obtained the type of protein completely different treatment SBG extract 2000 mg/kgbw and control. It shows that SBG extract 3000 mg/kgbw and 5000 mg/kgbw can stimulates the expression of new proteins that different from control mice.

Protein yield obtained showed that SBG extract can stimulate expression protein in the body of mice that have not can be identified. So that it can concluded that at a dose of 2000 mg/kgbw, 3000 mg/kgbw and 5000 mg/kgbw is not toxic in mice but can stimulate protein expression which is estimated to have a positive role for mice.

## 4. Conclusion

Based on the results of this study, it was concluded that SBG at a dose of 2000 mg/kgbw, 3000 mg/kgbw, 5000 mg/kgbw no show clinical signs of intoxication on Mus musculus such as motor activity and pupillary state normal, no convulsions (seizures), lacrimation, paralysis or mortality in mice and protein profile SBG parallel to 25 kDa BM marker tape.

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