The Effect of Paraquat Inhalation on Parkinsonism, Organ Morphology and Anatomy of Mice and Its Recovery with the Application of *Etliringea hemisphaerica* (Blume, R.M. Smith) Crude Extract

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Abstract. This research is aimed to understand the effect of paraquat herbicide inhalation on Parkinsonism, morphology and anatomy change in mice, and its recovery with *Etliringea hemisphaerica* crude extract application. Sixty mice were placed into three following groups: group R0 were mice receiving standard food ransom, R1 were a group of mice receiving the regular food ransom plus inhalation of 1% paraquat, and R2 were a group of R1 plus obtaining 0.39mg/bw extract *E. hemisphaerica* (Bl.) R.M. Smith). After 2 X 7 days of sub-sequential application of both paraquat and “helani tulip” extract, we observed the effects. The examination included bradikinesia attitude, postural instability and rigidity, morphology and anatomy of brain, liver, blood, lung, and kidney. The data were tabulated and analyzed qualitative and semi quantitative description on the behavioural disorder, the alteration of morphology and anatomy, and their remedy based on Sander 2004, Junqueira and Carneiro, 2007. The results showed that the application of paraquat caused strong bradikinesia, postural instability and rigidity. The treatment of the extract was only resulting in the bradikinesia removal but was minor improving the consequence of postural instability and rigidity. Paraquat was not affecting the morphology of neural brain but was altering the morphology and anatomy of lung, liver, blood, and the kidney. In general, the negative impact of the paraquat was weakly eliminated by the treatment of “helani tulip” extract.

Keywords: Anatomy change, *Etliringea hemisphaerica*, herbicide, inhalation, Paraquat, parkinsonism, recovery

1. Introduction
Paraquat is a toxic herbicide which is freely sold at the market. It is the most applicable in agriculture to eradicate unwanted wild plant in the crop growing field, but it is also the most poisonous chemical product used in undeveloped farming [1], and may strongly trigger a neurodegenerative Parkinsonism [2]. The Parkinsonism might be appeared after someone contaminated with heavy metal ion (Mn, Hg, Pb, etc), inhaled pesticide and or herbicide (paraquat, rotenone, dieldrin, and lindane), MPTP (a by-product of
synthetic opium), carbon monoxide (Anoxia), and post trauma syndrome [3]. The symptom of Parkinsonism is when anyone developed the abnormal tremor and shaky movement in hand, limbs, jaws, and facial muscle tissues [4]. Paraquat is one of the chemical compounds that may influence on the degeneration of neuron cells causes dementia and especially dopaminergic neuron-degeneration that might cause Parkinsonism and liver inflammation [5].

The study on herbal medication showed that some of the plant extract demonstrate the ability to recover and to prevent the Parkinsonism [6]. One of them is “helani tulip” or “honje hutan” (*Etlingera hemisphaerica* Blume, R.M. Smith) which contains an alkaloid, flavonoid, polyphenol, steroid, saponin, and atsiri oils. The extract may also include an antioxidant ascorbic acid to intoxicate the effect of the poisons [7].

Most of the Indonesian people are farmers and are still working disproportionately with the synthetic herbicide that might result in the occurrence of the Parkinsonism. Study with the application of the *Etlingera hemisphaerica* leaf extract has been able to recover Parkinsonism in mice that caused by mercury ion [8].

The injection application of paraquat on mice might have caused development of postural instability, rigidity and other parkinsonism phenomena and they are all displayed with the weak reflex movement avoiding the ravine, negative geotaxis reflex, reduction ability to swim [9]. The treatment with the *Etlingera* extract to the nauseous mice has been successfully rehabilitated the capability of smelling, seeing and hearing, and also the ability to lift up its body, although its improvement was slow.

As the inorganic farming system extensively uses paraquat that may cause the development of parkinsonism when it is inhaled via air respiration, we performed this investigation in order to understand whether the paraquat inhalation is affecting the development of parkinsonism in mice, and altering the morphology and anatomy of the lung, the liver, the blood, and the kidney, and whether the application of *Etlingera hemisphaerica* crude extract to the sick mice will remedy the nuisance.

2. Methods

We accomplished this research at our Laboratory during the period of June to September 2016. Preparation of the extract follows the work achieved by Gresinta, 2012 [10]. The leaf was finely ground, open drying and macerated on a shaker in the composition of one part of a fine crushed leaf and four parts of 96% ethanol. The maceration was taken place for a week. The macerate was separated with a filter so that we have a liquid crude extract. To get rid of the excess of ethanol, we evaporated it with a rotary evaporator, in a fifty degree Celsius of water bath. Finally, we have an extract in the form of pasta or a viscous liquid the “helani tulip” leaf crude extract.

We have sixty male mice placed in a proportional cage separated into three groups. The Group R0 were mice receiving standard pellet feed/ransom, R1 were a group of mice receiving the standard pellet ransom plus inhalation of 1% paraquat (*1,1-Dimetil-4,4-Bipyridium dichloride*) which has a brand product of proquat. The daily inhalation of paraquat has been carried on for seven days treatment respectively. The other groups were R2; these were a group of R1 receiving seven days of 1% paraquat and subsequently treated with obtaining 0.39 mg/gram of body weight “helani tulip” leaf extract (*Etlingrea hemisphaera*) for another seven days of applications. After fourteen days of all application, we observed the effects. The examination included the development of bradikinesia, the postural instability and rigidity, the alteration of the morphology and the anatomy of brain, liver, blood, lung, and kidney as a consequence of the paraquat inhalation. We also compared and evaluated the effect of the “helani tulip extract” treatments to the recovery of the annoyance.

The data contain qualitative and semi quantitative performance of the behavioural disorder, the alteration of the morphology and the anatomy influenced by paraquat inhalation, and their recovery after
the extract remedy. All the data were tabulated and analyzed descriptively. The comparative morphology and anatomy analysis uses Sander 2004, Junqueira and Carneiro, 2007.

The schemes of the treatments are as the following diagram:

![Diagram](image)

**Figure 1.** The scheme of the three group of treatments

### 3. Results and Discussion

#### 3.1 Effect of Paraquat on appearance of Parkinsonism

In general, as indicated in the corresponding tables, we can demonstrate that yes 1 % paraquat that was being inhaled to white mice has a significant result to the manifestation of Parkinsonism that is the development of bradikinesia, postural instability and rigidity.

#### Table 1. The effect of 1% paraquat inhalation and “helani tulip” leaf extract on bradikinesia.

| No | Observation | The result (response to the treatment) |
|----|-------------|---------------------------------------|
|    |             | Control (R0) | Paraquat (R1) | paraquat & extract (R2) |
| 1  | Bradikinesia | Normal       | Bradikinesia  | Inhaled into normal    |
3.2 The effect on postural instability and rigidity

Table 2. The effect of 1% paraquat inhalation and the treatment of “helani tulip” leaf extract to mice muscle performance (agility to avoid the artificial gap/ravine), geotaxis reflex, and the skill to swim (direction, position, and activity of swimming.

| The variety of observation | Treatment          |   |
|---------------------------|--------------------|---|
| Reflex to avoid gap       | Control            | Normal |
|                           | Paraquat (R1)      | Sharply decrease |
|                           | Paraquat & extract | Decrease |
| Reflex of Geotaxis        | Normal             | Sharply decrease |
|                           | Sharp decrease     | Decrease |
| Ability to swimming       | Normal             | Far Better |
| Swimming direction        | Normal             | Far Better |
| Swimming Position         | Normal             | Normal |
| Swimming Activity         | Normal             | Far Better |
|                           | Normal             | Not sufficiently good |

Table 3. The effect of 1% paraquat inhalation and the application of “helani tulip” leaf extract on the ability of smelling, seeing, and hearing, and lift up the body.

| No | Observation       | Control (R0) | Paraquat (R1) | paraquat and the extract |
|----|-------------------|--------------|---------------|--------------------------|
| 1  | Ability to smelling | Normal        | Disappear /bad| Not fully recovered       |
| 2  | Ability to seeing  | Normal        | Disappear /bad| Not fully recovered       |
| 3  | Ability to hearing | Normal        | Dropped sharply| Not fully recovered 100% |
| 4  | Ability to lift up the body | Normal | Drop sharply | Not fully recovered 100% |

Notes: Normal means having response 100% normal; Recovered to normal = having a positive response to the related challenge almost closed to normal (100%), dropped: positive response remain 50-99%, sharply dropped: positive response remains very low under 50%.
In general, the application of the extract was only resulting in the bradikinesia removal but was minor improving the consequence of postural instability and rigidity. The treatment of “helani tulip” leaf extract on Parkinsonism in mice has been able to fix or restored the bradikinesia in mice, but in general inadequately recovered the other bad appearance due to Parkinsonism. These are the ability to recover smelling, seeing and hearing capability, and also the capacity to lift up the body and its limbs back to the normal.
3.3 The effect of paraquat on the alteration of morphology and anatomy of selected organ, and its remedy affected by “the helani tulip” extract treatments

The following pictures showed the effects of Paraquat inhalation

![Figure 4](image-url)

**Figure 4.** The effect of control (R0), 1% paraquat (R1), and the paraquat and leaf “helani tulip” extract (R2) to the change of anatomy and morphology inner organ of mice.

The photo demonstrated the followings:

On mice organs control, the lungs display compactly pink in colour, whereas heart, liver, and kidney compactly old orange (R0); On mice organ of paraquat treatment, it is clear that its lungs showed compactly very pale pink, hearts have somehow swollen less densely orange, while the liver has exhibited barely pale as compare to the control and more enlarged and it seems due to inflammation (R1); On mice organ of paraquat treatment followed by leaf “helani tulip” extract it has shown that the lungs are very pale pink, the hearts still have inflammation with its colour remains a little bit recovered far close to the control, and the liver has recovered the colour, but it is still in the inflammation position (R2).

Paraquat was not affecting the morphology of neural brain but was strongly affecting on the morphology and anatomy of lung, liver, blood, and the kidney. In general, the applications of the “helani tulip” extract were weakly eliminating the negative influence of the paraquat.

Our result has yielded evidence on how the impact of paraquat treatment and its recovery with the application of the leaf “helani tulip” extract to the impairment effect of paraquat using mice as a model. In general, these results confirmed that the inhalation of 1% paraquat created the appearance of Parkinsonism. The behaviour test, mainly bradikinesia, postural instability, and tremor has indicated the above findings.

There is a significant effect of extract “helani tulip” leaf for the improvement of mice from bradikinesia caused by paraquat, but less significant impact for the repair of postural instability and tremor. Ones might expect that the recovery by the use of the extract needs to be longer in time periods.

In general, the treatment of 1% paraquat was not affecting the morphological appearance in the mice brain. The organ has specific compact reddish yellow in structure and colour. In fact, the central neuron in brain has much protection mechanism from any toxic external compounds, providing that the influence must be under the threshold value. It is because there is a barrier between blood vessel and the brain that its function is to filter any entrance of foreign molecules to the central neuron system. This mechanism is
to prevent the entry of unwanted toxic compound, including antibiotic and bacterial cells to the central neuron system in the brain [11]. Only in the overwhelming expose of the toxic compound, at the longer time exposure and followed by the weakness of immune system in our body, the possible access of the unwanted compound as paraquat and pathogen might penetrate the barrier. As a result, one might develop neurodegenerative syndrome as Parkinsonism appearance.

3.4 The effect of 1% paraquat inhalation to the morphological and anatomical lungs, hearts, liver, and kidney

As shown in the pictures, the lungs of mice control seem to have compactly pink or reddish pale in colour, while the heart, liver, and kidney have old orange in colour. When the 1% inhalation of paraquat was applied, it results in the lungs change the colour into being paler in pink colour, while the hearts have altered into old reddish orange and a little bit enlarges but less condensed due to the inflammation. The effect of paraquat inhalation to the livers and kidney showed to have paler and bigger as compared to the control in both livers, and kidneys.

The application of “helani tulip” extract for the recovery the mice organ from the impact paraquat inhalation is not fully accomplished. The lungs are still strongly pale, the hearts are still on enlarge because of inflammation, the colour of the liver has been close to normal, but it is yet in the swelling position.

These all are indications that the most in force of 1% paraquat inhalation are to the lungs organ. Histological structure of lungs that have the most influenced by 1% paraquat inhalation is parts of alveoli. This is because of the breathing the air containing the paraquat directly to the lungs may results in the direct impact of paraquat to the alveoli in the lungs. While the outer epithelial layer of pleura and connective tissue is shown to be normal. An aspect of inflammation is able to be depicted in our pictures, in which there is an enlargement of the alveoli volume.

The paraquat is strongly affecting to the quality of blood coagulation. Our histology pictures have confirmed the strong coagulation on control but weaker on paraquat treatment and relatively regained coagulation after the application extract of leaf “helani tulip”. The most probable of this occurrence is as a consequence of paraquat inhalation that preventing the blood coagulation.

3.5 The effect of 1% paraquat inhalation to the alteration of the kidney structure

In general, the weakness effect of 1% paraquat inhalation is to the inflammation of the kidney, and it is the least effect on structure kidney alteration.

We have observed the structure of cortex parts (and in part of medulla structures) of mice kidney. Investigation on both control and paraquat treatment suggested that the wall of the tissue of epithelium tubules are surrounding the parts of proximal tubules of the “contortus” cavity (P) and distal parts. The cavity of the P and D is brighter in control kidney rather than paraquat kidney. The structure of glomerulus (G) has shown to be spotted clustered, and the tissue of medulla parts is darker. This description is an indication that the structure of control kidney is more solid or compact, while the symptom of inflammation affected by paraquat is being demonstrated by the relative enlargement of P and D, and the structure of G is more protected.
3.6 The effect of paraquat inhalation to the alteration of liver histology

The effect of paraquat inhalation treatment to the histological structure of liver lobules of mice showed that the liver is experiencing an inflammation, which is relatively enlarged, and less condensed. The transparent hollow space is the venous vena surrounded by hepatocyte tissue which has formed like elongated tendrils enclosed by the sinusoid branch of ventral vena. The liver lobules from the control is relatively denser because of no inflammation, and therefore is more condensed and solid.

4. Conclusions

We have come to the following conclusions:

1. The application of 1% paraquat inhalation has brought about the male mice to experiencing the appearance of Parkinsonism, in which the bradikinesia, the postural instability and the tremor are some of the indications. The treatment of leaf “helani tulip” extract to the Parkinsonism in mice affected the recovery of bradikinesia but did not effect on other behaviour of Parkinsonism. The remedial treatment of “helani tulip” extracts needs to be the longer application to restore the deleterious effect of paraquat.

2. The application of paraquat inhalation has made relatively obvious to the morphology and anatomy of lungs, hearts, livers, and kidneys. The poisonous effect of paraquat inhalation on mice has being restored by the application of leaf “helani tulip” extract, and the restoration of morphology and anatomy of lungs, liver, hearts and kidney are some of the indications.

3. The application of paraquat inhalation did not result in the alteration of brains morphology, and the histology of central neural tissues of mice.

4. The application of paraquat inhalation, in general, decreased the ability of blood coagulation. While the application of leaf “helani tulip” extract relatively recovered the reduction coagulation ability caused by paraquat inhalation.

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