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# Evaluation of antiparkinsonian activity of water yam tuber (*Dioscorea alata* L.) extract on haloperidol-induced Parkinson's disease in mice

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## ABSTRACT

Parkinson's disease (PD) appears as motor dysfunction that is attributed to depleting dopamine levels due to cell death in the extrapyramidal system that is comprised of the basal ganglia's motor neurons. Oxidative stress is central in triggering cell death. Water yam (*Dioscorea alata* L.) contains anthocyanins with potential antioxidative and neuroprotective activities that can ameliorate oxidative stress in PD. This research aimed to assess the antiparkinsonian activity of the water yam's ethanol extract by observing motor (bar test, rotarod test, negative geotaxis reflex test, cliff avoidance test) and sensory activities (olfactory testing) in vivo in mice with haloperidol-induced PD. Water yam tubers were extracted by maceration with the solvent 70% ethanol. This experimental research employed a posttest-only control group design where 35 mice were equally distributed into 7 treatment groups, containing 5 each: Group 1 (normal group) was administered Carboxymethyl Cellulose Sodium (CMC-Na) orally (p.o.) and aqua pro injection intraperitoneally (i.p.); Group 2 (negative group) was given CMC-Na p.o.; Groups 3 and 4 (positive groups) received, respectively, levodopa p.o. and curcumin p.o.; and then, finally, Groups 5, 6, and 7 (test groups) were given the water yam extracts at varying doses: 100, 200, and 400 mg/kg BW. CMC-Na, levodopa, and the extract were given once a day for 7 days. Fifteen minutes after receiving their respective treatments on the last day, all the test animals, except for Group 1, were injected with haloperidol solution at 2 mg/kg BW i.p. Then, a bar test, rotarod test, geotaxis reflex test, olfactory testing, and cliff avoidance test were performed to examine their motor and sensory responses in the 5, 60, 120, and 180th minutes. The data obtained were inputted and analyzed statistically with a One-Way ANOVA and then continued with an LSD test. In addition, thin-layer chromatography was employed as the anthocyanin screening test for the ethanol extract of water yam tubers. Results showed that the ethanol extract contained pelargonidin, and when given at 200 and 400 mg/kg, it substantially shortened the exploration time and prolonged latency to first fall in all the tests relative to the haloperidol, levodopa, and curcumin groups ($p<0.05$). Thus, it can be inferred that the ethanol extract of water yam contains pelargonidin (an anthocyanin compound) and has the activity of preventing haloperidol-induced PD in mice when administered at 200 and 400 mg/kg BW.

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**Keywords**: *Dioscorea alata* L., haloperidol, levodopa, curcumin, parkinson

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## INTRODUCTION

Parkinson's disease (PD) is manifested in impaired motor functions in response to decreased dopamine levels due to cell death in the extrapyramidal system that is comprised of the basal ganglia's motor nerurons. PD is complex idiopathic neurological disorder associated with age that is the second-most common after Alzheimer's (DeMaagd & Philip, 2015b). PD shows a rapid increase in prevalence, and in 2019, it caused an estimated 329,000 deaths and 5.8 million disability-adjusted life years (DALYs) (World Health Organization., 2022). Several main symptoms of PD are tremors, bradykinesia, and muscle stiffness, along with postural instability and gait impairment. Striatal dopamine depletion and deficiency of dopaminergic neurons in the pars compacta (SNpc) are the two pathologic signs of PD. PD mostly progresses in these mechanisms of excitotoxicity, failures in the pathways for protein clearance, mitochondrial dysfunction, mutation, neuroinflammation, oxidative stress, and protein aggregation (Maiti et al., 2017). In addition, epidemiological evidence suggests that chronic exposure to environmental pollutants, including rotenone as an inhibitor of complex I, increases the risk of developing PD (Strathearn et al., 2014).

Levodopa is the mainstay of treatment for PD's motor symptoms but has several documented side effects. Levodopa can cross the blood-brain barrier and is then metabolized to dopamine by L-amino acid decarboxylase (DDC). The most frequently reported side effects linked to levodopa are confusion, dark urine, dizziness, hypotension, nausea to vomiting, sedation, unusual sex drive, and vivid dreams, which are often problematic, especially in elderly patients (DeMaagd & Philip, 2015a).

Water yam (Dioscorea spp.) is consumed in several parts of the world. It contains numerous bioactive compounds known to have health benefits, such as diosgenin, dioscin, dioscorin, and anthocyanins. It has been scientifically documented that the extract has antihypertensive, antioxidant, antimicrobial, immunomodulatory activities, and estrogenic effects (Srivichai & Hongsprabhas, 2020). Some anthocyanins found in water yams are delphinidin-3,5-diglucoside, cyanidin-3,5-diglucoside, delphinidin-3-glucoside, cyanidin-3-diglucoside, delphinidin-3-glucose-5-rutinoside, and cyanidin 3-(6-sinapoyl gentiobioside) (Adomėnienė & Venskutonis, 2022). Anthocyanin-rich extracts have more potent neuroprotective activity than those containing other polyphenols at high concentrations. Previous studies proved anthocyanins interfere with rotenone neurotoxicity (Strathearn et al., 2014). Neuroprotective activity is closely related to lowered ROS levels in PD (Carrera & Cacabelos, 2019). The research objective was to assess the pharmaceutical activity of the water yam tuber's ethanol extract that contained anthocyanins with in vivo measurements using a mouse model of haloperidol-induced PD.

## MATERIALS AND METHODS

### Materials

This research used water yams (*Dioscorea alata* L.) (tuber parts) obtained from Madiun (Indonesia), which had been authenticated by the Biology Laboratory, Faculty of Applied Science and Technology, Ahmad Dahlan University (Nr. 438/Lab. Bio/B/X/2022). Other materials were 70% ethanol from General Labora (Indonesia), Carboxymethyl Cellulose Sodium (CMC-Na) from Brataco (Indonesia), distilled water from Jaya Sentosa (Indonesia), haloperidol from OGB Dexa (Indonesia), levodopa from Mepro (Indonesia), and 0.9% sodium chloride (NaCl) from Widarta Bakti (Indonesia). Curcumin, ammoniac, acetic acid, concentrated hydrochloric acid (HCl), and formic acid were from Merck KGaA (Germany).

### Methods

#### Water yam tuber extraction

Ten kg of water yam tubers were peeled, washed, thinly sliced, covered with a dark cloth for drying under the sun, and then ground to form a powder. The tuber powder was weighed and macerated at room temperature (±25°C) with 70% ethanol (1:10 w/v) for 5 mins, with ethanol comprising 75% of the total volume and stirring once a day. Ethanol was used as a solvent because it can extract antioxidant compounds with good results and is safe for natural solvents (Hikmawanti et al., 2021). After 5 days,

the ethanol extract was filtered using a filter cloth and collected in a glass jar. The retained material was macerated with the remaining 25% volume of ethanol (v/v) for 2 days. After the re-maceration, the results were filtered and combined with the previous maceration product, which was later evaporated at 50°C to remove the ethanol content from the extract using a rotary evaporator from Heidolph (Germany) until a thick extract was obtained. To achieve the research objective, 0.5% CMC-Na was used to dissolve the concentrated extract to prepare a carrier for suspensions to be delivered at the desired doses: 100, 200, and 400 mg/kg (Christina & Rifa'i, 2014).

**Qualitative identification of anthocyanins using thin layer chromatography (TLC)**

Before the TLC test, acid hydrolysis was conducted to obtain anthocyanidin extract. Acid hydrolysis started with heating the tuber extract in 2M HCl for 40 mins, then cooling and washing with ethyl acetate twice. Next, the ethyl acetate fraction was disposed of, and then the remaining ethyl acetate was removed from the water fraction by heating at 80°C for 3 mins. Afterward, the water fraction was extracted with amyl alcohol, and the amyl alcohol fraction was concentrated in a watch glass over a boiling water bath. The dried anthocyanidin extract was dissolved in ± 1 ml of 0.01% HCl that contained methanol. In the thin-layer chromatography (TLC), the anthocyanidin extract was spotted on a cellulose plate and then allowed to dry. Afterward, the plate was developed in one direction with the mobile phases forestal (acetic acid:concentrated HCl:H2O, 30:3:10, v/v) and formate (formic acid:concentrated HCl:H2O, 5:2:3, v/v). Finally, the derived Rf value and color (observed under visible and UV lights) of each appearing spot were compared with the Reference Table (Lestario, 2017).

**In-vivo screening of anti-parkinson activity of water yam tuber extract**

This study used male mice of the Deutschland, Denken, and Yoken (DDY) strain aged 2 months and weighing 30–40 g, acquired from the Integrated Research and Testing Laboratory (LPPT), Universitas Gadjah Mada (UGM). Prior to the experiment, the test mice were acclimatized for 7 days to laboratory conditions: 25–26ºC temperature, 50–60% humidity, exposure to a 12:12h light-dark (LD) cycle, and allowed ad libitum feeding and water intake. Afterward, they were separated into 7 treatment groups, each consisting of 5 mice:

Normal group: given CMC-Na per oral (p.o.) and aqua pro injection intraperitoneally (i.p.)
Negative control group: CMC-Na (p.o.) and haloperidol i.p. at the dose of 2 mg/kg BW
Positive control group 1: levodopa at 39 mg/kg BW (p.o.) and haloperidol i.p.
Positive control group 2: curcumin at 208 mg/kg BW (p.o.) and haloperidol i.p.
Test group 100: water yam extract at 100 mg/kg BW and haloperidol i.p.
Test group 200: water yam extract at 200 mg/kg BW and haloperidol i.p.
Test group 400: water yam extract at 400 mg/kg BW and haloperidol i.p.

Stock solutions of CMC-Na (0.5% w/v), haloperidol (0.5 mg/mL v/v), levodopa (3 mg/mL v/v), and water yam extract at 40 mg/mL v/v were prepared before the treatment. CMC-Na, levodopa, and the extract were given once a day for 7 days. The administered volume was adjusted to the mouse's weight using the formula 1.

$$Volume\ of\ administration = Mouse\ weight\ (kg) \times \frac{Administration\ dose\ (kg\ BW)}{Stock\ solution\ concentration\ (^{mg}/_{mL})} \quad (1)$$

Haloperidol was selected as the negative control because it reduces the production of plasma dopamine (Nofitasari et al., 2019). Curcumin was chosen as the second positive control because it has been shown to have neuroprotective activity in PD (Nebrisi, 2021). The extract doses were selected based on Bagewadi & Khan's research in 2015, which proved that significantly different doses between groups produced results with notable differences. Fifteen minutes after receiving their respective treatments on the last day, all the test animals, except the normal group, were injected with haloperidol at 2 mg/kg BW i.p. Then, the activity testing, comprising bar test, rotarod test, negative geotaxis reflex

test, olfactory test, and cliff avoidance test, was conducted in the 5, 60, 120, and 180th minutes (Bagewadi, 2015). This research received ethical approval from the Research Ethics Committee of Universitas Ahmad Dahlan (Nr. 012207095).

**Bar test**

The bar test aimed to determine motor ability by observing the catalepsy time of the tested mouse. The mouse was gently positioned with both of its forelimbs raised and resting on a wooden bar with a 0.9-cm diameter set to 3 cm high above the surface. This step marked the beginning of the time count, which stopped when the mouse removed both paws from the bar or its head moved around as a sign of exploring. This length of time was recorded as the catalepsy time. The maximum time used for catalepsy measurements is 720 s. Typically, mice have a short catalepsy time (Bagewadi, 2015).

**Rotarod test**

In addition to the bar test, the motor ability was also examined using the rotarod test to measure the latency to the first fall (endurance). The mouse was placed on a rod, which was set to rotate at ±10 rotations per minute (rpm). The duration for when the mouse remained on and endured the rotarod and the rotating speed at which the mouse first fell were recorded. Usually, mice can maintain their balance unlimitedly (Lubrich et al., 2022).

**Negative geotaxis test**

The negative geotaxis reflex aimed to determine mice's motor and sensory abilities as a response to geo-gravitational stimuli and to measure sensorimotor competence. The mouse was put on a platform (12.5 cm in length x 45 cm in height) that were covered with a coarse cloth paper and set at 45° inclination. The length of time that the mouse spent rotating 180° was measured, which is normally short in mice (Ruhela et al., 2019).

**Olfactory testing**

Olfactory testing aimed to assess sensory ability by observing the response of the tested mouse to an unpleasant odor (ammonia). The mouse was held still, and the nose was brought close to a cotton bud dipped in ammonia. A positive result occurs when the mouse avoids the cotton bud, whereas a negative is when it stays still or does not react to the sharp odor of ammonia. Mice with the normal olfactory function will immediately pull their nose away from the ammonia-dipped cotton bud (Nababan et al., 2015).

**Cliff avoidance test**

The cliff avoidance test was intended to evaluate motor ability based on the mouse's response to cliffs. The test mouse was placed at the edge of a flat table with its nose and forelimbs parallel to it. Then, its reaction to the table end or "cliff" was observed, and the time it spent to avoid or pull away from the cliff was recorded. Normally, a mouse immediately pulls itself away when brought closer to the edge of a cliff/table (Xie et al., 2020).

**Data Analysis**

Data recorded during the motor and sensory ability testing were analyzed statistically with the Shapiro-Wilk normality test, followed by a one-way ANOVA. Afterward, an LSD test was performed to see differences between the groups at each time of observation. The statistical analyses were run in the SPSS program, and statistically significant results would be indicated by a p-value of less than 0.05.

## RESULT AND DISCUSSION

## RESULTS

### *Anthocyanidin qualitative Test with TLC*

Thin-layer chromatography (TLC) testing was conducted by looking at the RF value and visible color. Table 1 shows that the ethanol extract of the water yam tuber is suspected of containing pelargonidin. Pelargonidin is the most common anthocyanidin and anthocyanin distributed in plants besides cyanidin, delphinidin, peonidin, malvidin, and petunidin (Khoo et al., 2017).

**Table 1. Thin-layer chromatography results of the water yam extract**

| Pigment | Rf (×100) in [standard] | | Visible color [standard] | Rf (×100) in [result] | | Visible color [result] | Conclusion |
|---|---|---|---|---|---|---|---|
| | Forestal | Formic | | Forestal | Formic | | |
| Pelargonidin | 68 | 33 | Red | 68 | 59 | Red | Suspected of containing pelargonidin |
| Cyanidin | 49 | 22 | Magenta | | | | |
| Peonidin | 63 | 30 | Magenta | | | | |
| Delphinidin | 32 | 13 | Purple | | | | |
| Petunidin | 46 | 20 | Purple | | | | |
| Malvidin | 60 | 27 | Purple | | | | |

Notes: Forestal = Acetic acid : Conc. HCl : Water (3:30:10); Formic = Formic acid : Conc. HCl : Water (5:2:3)

### *In-vivo anti-parkinson activity of the water yam tuber extract*

Relative to the normal group, a statistically significant extension of the catalepsy time obtained from the bar test for the negative control group (haloperidol) ($p<0.05$) was observed in the 5, 60, 120, and 180th minutes. Also, the catalepsy time of the two positive groups (levodopa and curcumin) decreased significantly ($p<0.05$) in these minutes or in contrast to the negative group. The test groups receiving 200 and 400 mg/kg BW of the water yam extract also demonstrated a substantial reduction in catalepsy time in the 60, 120, and 180th minutes ($p<0.05$), which was significantly different from the groups treated with haloperidol, levodopa, and curcumin, as shown in Table 2.

**Table 2. Mean catalepsy time in the bar test of mice with haloperidol-induced Parkinson's disease that were given different doses of water yam tuber (Dioscorea alata L.) extract (in mean±SD, s)**

| Group | Catalepsy time in the *i*-th minute | | | |
|---|---|---|---|---|
| | 5 | 60 | 120 | 180 |
| Normal | $0.77 \pm 0.15^{bcd}$ | $0.81 \pm 0.10^{bcd}$ | $0.59 \pm 0.10^{bcd}$ | $0.72 \pm 0.09^{bcd}$ |
| Haloperidol | $2.83 \pm 1.33^{acd}$ | $30.97 \pm 5.65^{acd}$ | $61.59 \pm 1.31^{acd}$ | $72.05 \pm 2.32^{acd}$ |
| Levodopa | $4.00 \pm 0.73^{ab}$ | $21.83 \pm 1.40^{ab}$ | $41.50 \pm 1.47^{abd}$ | $62.78 \pm 2.32^{abd}$ |
| Curcumin | $3.37 \pm 0.49^{a}$ | $22.40 \pm 1.95^{ab}$ | $31.18 \pm 2.52^{abc}$ | $18.04 \pm 1.13^{abc}$ |
| Extract 100 mg/kg BW | $2.99 \pm 0.57^{ac}$ | $28.35 \pm 2.38^{acd}$ | $44.02 \pm 10.62^{abd}$ | $32.77 \pm 5.26^{abcd}$ |
| Extract 200 mg/kg BW | $3.12 \pm 1.29^{a}$ | $17.08 \pm 2.72^{abcd}$ | $25.28 \pm 2.08^{abcd}$ | $27.94 \pm 1.82^{abcd}$ |
| Extract 400 mg/kg BW | $1.91 \pm 0.59^{acd}$ | $27.40 \pm 2.78^{abcd}$ | $25.33 \pm 2.24^{abcd}$ | $25.75 \pm 2.13^{abcd}$ |

Notes: the superscript letters denote a statistically significant difference from the normal group (*a*), the haloperidol group (*b*), the levodopa group (*c*), and the curcumin group (*d*)

In the negative control group (haloperidol), a pronounced reduction in latency time to the first fall was observed in the 5, 60, 120, and 180th minutes, which was significantly different from the normal group ($p<0.05$). In contrast, the latency time of the two positive groups (levodopa and curcumin) in these minutes considerably increased or were significantly different from the negative group ($p<0.05$). Furthermore, receiving 100 and 200 mg/kg BW of the extract, the two test groups exhibited a

significantly longer latency time in all the observation minutes ($p<0.05$), relative to the groups treated with haloperidol and levodopa, as shown in Table 3.

**Table 3. Mean latency time to first fall in the rotarod test of mice with haloperidol-induced Parkinson's disease that were given different doses of water yam tuber (Dioscorea alata L.) extract (in mean±SD, s)**

| Group | Latency time to fall in the *i*-th minute | | | |
|---|---|---|---|---|
| | 5 | 60 | 120 | 180 |
| Normal | $26.91 \pm 6.62^{bcd}$ | $31.80 \pm 11.48^{b}$ | $43.17 \pm 13.44^{bcd}$ | $54.62 \pm 14.61^{bcd}$ |
| Haloperidol | $10.03 \pm 1.51^{acd}$ | $8.73 \pm 1.63^{acd}$ | $11.14 \pm 4.75^{acd}$ | $11.79 \pm 4.92^{acd}$ |
| Levodopa | $20.95 \pm 3.95^{ab}$ | $26.99 \pm 5.91^{b}$ | $29.20 \pm 7.16^{ab}$ | $34.26 \pm 7.67^{abd}$ |
| Curcumin | $18.76 \pm 1.76^{ab}$ | $26.45 \pm 3.89^{b}$ | $29.15 \pm 3.86^{ab}$ | $31.27 \pm 2.05^{ab}$ |
| Extract 100 mg/kg BW | $14.56 \pm 3.88^{ac}$ | $18.93 \pm 8.72^{abcd}$ | $21.08 \pm 7.52^{ab}$ | $25.84 \pm 7.02^{ab}$ |
| Extract 200 mg/kg BW | $15.79 \pm 3.27^{abc}$ | $15.79 \pm 3.27^{abc}$ | $15.79 \pm 3.27^{abc}$ | $15.79 \pm 3.27^{abc}$ |
| Extract 400 mg/kg BW | $23.21 \pm 3.73^{ab}$ | $23.21 \pm 3.73^{ab}$ | $23.21 \pm 3.73^{ab}$ | $23.21 \pm 3.73^{ab}$ |

Notes: the superscript letters denote a statistically significant difference from the normal group (*a*), the haloperidol group (*b*), the levodopa group (*c*), and the curcumin group (*d*)

In the negative control group (haloperidol), the half-turn time was significantly longer in all the observation periods than the normal group ($p<0.05$). Contrary results appeared in the two positive groups (levodopa and curcumin) as the half-turn time shortened significantly in the 60, 120, and 180th minutes ($p<0.05$), compared to the negative group. In the 100 and 200 mg/kg BW extract groups, the half-turn time substantially shortened in the 60, 120, and 180th minute, which was significantly different ($p<0.05$) from the groups treated with haloperidol, levodopa, and curcumin, as presented in Table 4.

**Table 4. Mean half-turn time (180° rotation) in the negative geotaxis test of mice with haloperidol-induced Parkinson's disease that were given different doses of water yam tuber (Dioscorea alata L.) extract (in mean±SD, s)**

| Group | Mean half-turn time in the *i*-th minute | | | |
|---|---|---|---|---|
| | 5 | 60 | 120 | 180 |
| Normal/Healthy | $2.71 \pm 1.93^{bcd}$ | $2.72 \pm 0.72^{bcd}$ | $4.36 \pm 1.17^{bcd}$ | $8.09 \pm 1.68^{bcd}$ |
| Haloperidol | $23.20 \pm 0.68^{acd}$ | $34.48 \pm 0.78^{acd}$ | $47.03 \pm 0.64^{acd}$ | $58.37 \pm 1.46^{acd}$ |
| Levodopa | $36.98 \pm 0.47^{abd}$ | $24.37 \pm 1.08^{abd}$ | $13.25 \pm 0.92^{abd}$ | $48.45 \pm 1.38^{abd}$ |
| Curcumin | $30.22 \pm 1.45^{abc}$ | $16.97 \pm 0.73^{abc}$ | $10.34 \pm 1.36^{abc}$ | $20.54 \pm 1.59^{abc}$ |
| Extract 100 mg/kg BW | $25.90 \pm 1.83^{ac}$ | $17.88 \pm 1.12^{abc}$ | $11.55 \pm 1.38^{ab}$ | $13.18 \pm 1.42^{abcd}$ |
| Extract 200 mg/kg BW | $38.72 \pm 5.68^{abd}$ | $17.48 \pm 2.04^{abc}$ | $12.85 \pm 1.55^{abd}$ | $30.27 \pm 4.20^{abcd}$ |
| Extract 400 mg/kg BW | $55.89 \pm 13.31^{abcd}$ | $25.05 \pm 3.23^{abd}$ | $34.08 \pm 2.72^{abcd}$ | $47.07 \pm 7.10^{abd}$ |

Notes: the superscript letters denote a statistically significant difference from the normal group (*a*), the haloperidol group (*b*), the levodopa group (*c*), and the curcumin group (*d*)

In the negative control group (haloperidol), the olfactory reflexes were increasingly longer ($p<0.05$) in all the observation periods, which were significantly different from the normal group. Relative to the negative group, the two positive groups (levodopa and curcumin) showed a significantly shortening reflex time ($p<0.05$) in the 5, 60, 120, and 180th minutes. Similarly, in these minutes, the 200 and 400 mg/kg BW extracts produced a decrease in the reflex time, which were significantly different from the group receiving haloperidol ($p<0.05$), as shown in Table 5.

In the cliff avoidance test, the negative control group (haloperidol) required an increasingly longer time to react during the entire observation, which was significantly different from the normal group. On the contrary, the two positive groups (levodopa and curcumin) showed significantly short reaction time in the 5, 60, 120, and 180th minutes compared to the negative group ($p<0.05$). Similarly, the 200 and 400 mg/kg BW extract groups also exhibited significant shortening in the reaction time in the 60, 120, and 180th minutes compared to the haloperidol and levodopa groups ($p<0.05$), as shown in Table 6.

**Table 5. Olfactory testing results of mice with haloperidol-induced Parkinson's disease that were given different doses of water yam tuber (Dioscorea alata L.) extract (in mean±SD, s)**

| Group | Olfactory reflex time in the *i*-th minute | | | |
|---|---|---|---|---|
| | 5 | 60 | 120 | 180 |
| Normal/Healthy | 3.06 ± 1.37$^{b}$ | 2.30 ± 0.70$^{b}$ | 2.74 ± 1.02$^{bc}$ | 3.91 ± 1.93$^{b}$ |
| Haloperidol | 15.17 ± 2.14$^{acd}$ | 26.42 ± 0.81$^{acd}$ | 35.44 ± 5.39$^{acd}$ | 43.92 ± 5.21$^{acd}$ |
| Levodopa | 2.02 ± 0.53$^{b}$ | 2.63 ± 0.37$^{b}$ | 5.61 ± 0.76$^{ab}$ | 4.11 ± 0.55$^{b}$ |
| Curcumin | 1.77 ± 0.23$^{b}$ | 2.79 ± 0.47$^{b}$ | 3.38 ± 0.94$^{b}$ | 2.67 ± 0.54$^{b}$ |
| Extract 100 mg/kg BW | 5.17 ± 2.60$^{abcd}$ | 12.09 ± 1.87$^{abcd}$ | 13.84 ± 2.38$^{abcd}$ | 3.43 ± 1.91$^{b}$ |
| Extract 200 mg/kg BW | 5.22 ± 0.87$^{abcd}$ | 7.35 ± 0.86$^{abcd}$ | 8.35 ± 0.57$^{abcd}$ | 3.19 ± 0.65$^{b}$ |
| Extract 400 mg/kg BW | 2.44 ± 0.95$^{b}$ | 4.32 ± 0.55$^{abcd}$ | 1.65 ± 0.41$^{bc}$ | 1.19 ± 0.18$^{abc}$ |

Notes: the superscript letters denote a statistically significant difference from the normal group (*a*), the haloperidol group (*b*), the levodopa group (*c*), and the curcumin group (*d*)

**Table 6. Cliff avoidance test results of mice with haloperidol-induced Parkinson's disease that were given different doses of water yam tuber (Dioscorea alata L.) extract (in mean±SD,s)**

| Group | Reaction time in the *i*-th minute | | | |
|---|---|---|---|---|
| | 5 | 60 | 120 | 180 |
| Normal | 9.00 ± 0.93$^{bcd}$ | 9.11 ± 0.97$^{bcd}$ | 9.17 ± 0.54$^{bcd}$ | 8.31 ± 1.01$^{bc}$ |
| Haloperidol | 44.17 ± 3.17$^{acd}$ | 47.28 ± 3.63$^{acd}$ | 55.24 ± 5.21$^{acd}$ | 51.93 ± 5.17$^{acd}$ |
| Levodopa | 17.82 ± 4.55$^{ab}$ | 27.10 ± 4.39$^{ab}$ | 29.08 ± 4.60$^{ab}$ | 22.68 ± 9.60$^{abd}$ |
| Curcumin | 15.28 ± 1.32$^{ab}$ | 24.34 ± 4.02$^{ab}$ | 26.05 ± 7.32$^{ab}$ | 12.86 ± 1.85$^{bc}$ |
| Extract 100 mg/kg BW | 17.88 ± 3.90$^{ab}$ | 32.87 ±17.96$^{ab}$ | 34.27 ± 11.51$^{abd}$ | 26.58 ± 12.37$^{abd}$ |
| Extract 200 mg/kg BW | 16.50 ± 6.25$^{ab}$ | 18.37 ± 1.48$^{abc}$ | 18.43 ± 2.26$^{abcd}$ | 11.24 ± 2.06$^{bc}$ |
| Extract 400 mg/kg BW | 13.92 ± 2.93$^{ab}$ | 15.13 ± 1.35$^{bcd}$ | 16.01 ± 1.65$^{bcd}$ | 10.76 ± 1.41$^{bc}$ |

Notes: the superscript letters denote a statistically significant difference from the normal group (*a*), the haloperidol group (*b*), the levodopa group (*c*), and the curcumin group (*d*)

## DISCUSSION

Parkinson's disease (PD) occurs as an extrapyramidal disorder manifested in the motor structures of the pars reticulata (SNpc) and the basal ganglia, which are parts of larger circuits situated in both thalamus and cortex. In PD patients, striatal dopamine depletion increases the activity in the globus pallidal circuit (GPi) and SNpc, leading to the impaired functioning of gamma-aminobutyric acid (GABA) and, consequently, thalamic inhibition. These chain effects diminish the thalamus' capacity to initiate the frontal cortex, decreasing motor and sensory activity (DeMaagd & Philip, 2015b).

One theory about the underlying cause of PD is oxidative stress. Oxidative stress refers to a condition of too much oxygen in the environment, which can damage cells in the brain of patients with PD. Some brain chemicals, such as dopamine and serotonin, are known antioxidants. However, dopamine can form reactive oxygen species when cleaved jointly by catechol-O-methyltransferase (COMT) and monoamine oxidase-B (MaO-B). Increased levels of oxidative stress markers have been found in PD patients, as evidenced by damage to DNA, protein, and fat. Therefore, it is possible that inhibiting MaO-B and COMT activity can help restore dopamine and treat PD (Ishiki et al., 2018).

Results showed that haloperidol (an antipsychotic drug) decreased motor and sensory functions. The motor function testing included catalepsy time on the bar test, number of falls and latency to first fall (as observed in the rotarod test), geotactic reflex, and reaction time to avoid the cliff. Sensory functions were examined from the ability to smell, in which haloperidol was found to reduce the production of plasma dopamine levels because it is an antipsychotic drug whose mechanism of action involves inhibiting striatal postsynaptic dopamine receptors. This effect causes extrapyramidal symptoms, e.g., catalepsy (muscle stiffness), mediated by striatal D2 receptor blockade (Nofitasari et al., 2019).

In addition, it was found that levodopa improved haloperidol-induced motor and sensory impairments in mice. Levodopa is a prodrug of dopamine, meaning it has the ability to traverse the blood-brain barrier to be subjected to further metabolization into dopamine. It is also extensively metabolized in the digestive tract, and a drug called carbidopa is given to treat this. In the pars compacta, dopa decarboxylase converts levodopa to dopamine. It is then deposited in the presynaptic neuron and, when needed, secreted into the synaptic cleft to bind to postsynaptic dopamine D1 and D2 receptors (DeMaagd & Philip, 2015a).

Besides levodopa, curcumin proves effective in preventing motor and sensory impairments caused by haloperidol due to its capacity to regulate the function of several signal transduction pathways. Moreover, curcumin can increase dopamine levels and availability in the brain, contributing to its neuroprotective effect. Previous research on animal models of 6-OHDA found that this substance increases the survival of tyrosine hydroxylase fibers and SNpc neurons in the striatum, reduces behavioral abnormalities like relapses, and exerts neuroprotective effects through α7-nAChR- mediated mechanisms (Nebrisi, 2021).

Water yam extracts could improve the symptoms of motor and sensory dysfunctions in mice treated with haloperidol. Based on the identification results, the extract is suspected of containing an anthocyanin compound called pelargonidin. Also, depending on the administered dose, it has different effects on the motor and sensory tests. For instance, in the bar test, olfactory testing, and cliff avoidance test, the test groups receiving 200 and 400 mg/kg BW of the water yam tuber extract showed favorable improvements throughout the observation, i.e., prolonged catalepsy time and reduced reflex/reaction time. Meanwhile, in the rotarod and negative geotaxis tests, the extract was effective at 100 and 200 mg/kg BW. Differences in these results are presumably caused by subjecting the test animals to five tests in one time span. Therefore, it can be concluded that the water yam extract will have the potential as antiparkinsonian when administered at 200 or 400 mg/kg BW, which corresponds to the dose-response curve hypothesis. This hypothesis states that the higher the dose given, the greater the pharmacological effect produced (Farinde, 2022).

Anthocyanins can reduce PD's symptoms due to their neuroprotective characteristics. The primary causes of oxidative stress under pathophysiological conditions of PD are neuroinflammation of dopaminergic neurons, mitochondrial dysfunction, and dopamine metabolism. Anthocyanins counteract oxidative stress on nerves, thus exerting a neuroprotective effect on PD (Ullah et al., 2019). In terms of neuroprotection, anthocyanins work by reducing intracellular ROS levels in the neuroblastoma cells SK-N-SH. Furthermore, this flavonoid also inhibits the activation of the apoptosis signal-regulating kinase 1 (ASK1)–JNK/p38 pathway that is ROS-dependent, the regulated expression of the NEU1 gene or sialidase-1, and stimulation of heme oxygenase-1 expression (Khoo et al., 2017).

## CONCLUSION

The ethanol extract of water yam tuber that contains pelargonidin is a potential antiparkinsonian. At 200 and 400 mg/kg BW doses, this extract can prevent motor and sensory impairments in mice with haloperidol-induced Parkinson's disease.

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