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BIOSYNTHESIS, CHARACTERIZATION AND ANTIPARKINSON ACTIVITY OF MAGNETITE-INDONESIAN VELVET BEANS (*Mucuna pruriens* L.) NANOPARTICLES

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Abstract

The aims of the present study were to synthesize magnetite nanoparticles mediated by the extract of velvet bean (*Mucuna pruriens*) seed from Indonesia and evaluate its anticataleptic effect on haloperidol-induced catalepsy mice. The magnetite-*M. pruriens* seed extract nanoparticles (FeMPn) were synthesized by reacting ferric chloride ($FeCl_3$) solution with *M. pruriens* seed extract. Scanning Electron Microscopy-Energy Dispersive X-ray (SEM-EDX), Transmission Electron Microscopy (TEM), Fourier-Transform Infrared (FTIR) and Thermogravimetric Analysis/Differential Thermal Analysis (TGA/DTA) were used to characterize the FeMPn synthesized. Catalepsy test of FeMPn was performed at dosages of 5, 10, 15, 20 and 25 mg/kg body weight. SEM and TEM images showed the aggregation of FeMPn with a spherical shape and smallest size of 30.5 nm. FTIR spectra confirmed the presence of Fe-O bonding from the absorption band at 557.4 cm^{-1}. TGA/DTA results confirmed that the addition of Fe leads to increase the thermal stability of the extract. The administration of FeMPn at all dosages was able to lower the catalepsy in mice significantly and it was better than the extract itself with the best dosage was 10 mg/kg body weight. These findings suggest that FeMPn could be a promising option for antiparkinson treatment.

Keywords: Catalepsy, Extract, Magnetite, *Mucuna pruriens*, Nanoparticle.

1. Introduction

The second most common progressive neurodegenerative disease and mostly affects people over the age of 60 is Parkinson [1]. Of the various symptoms emerged in Parkinson's disease, the disease is closely related to the damage of brain nerve cell in the substantia nigra pars compacta that serve to release dopamine, which ultimately leads to a decrease in dopamine levels [2-5]. Dopaminergic neurotransmission is the first step in signalling of motor activities. Consequently, the decrease in dopamine levels makes people with Parkinson lose control of motor activity [1]. The symptoms of the disease include progressive loss of muscle control, slowness of movements (bradykinesia), tremor, rigidity and postural abnormalities called catalepsy [3, 5, 6]. Sanberg et al. [7] and Wilcox and Duffy [8] commented that catalepsy is a neurological condition where the muscles show the rigidity and fixity, thus the patient will tend to retain an imposed posture for a prolonged period of time. The most widely used synthetic drug in Parkinson's treatment is Levodopa, while carbidopa or benserazide usually use as a drug combination to increase the therapeutic benefit [6, 9]. Unfortunately, motor complications often arise in long-term use in addition to other side effects such as digestive disorders, sleep disorders, liver and kidney disorders, dyskinesia, hallucinations, visual disorders and depression [6]. Therefore, it is necessary to find an alternative treatment for Parkinson's disease, which is not only safer but also effective.

Mucuna pruriens or velvet bean is reported to be used in the treatment of Parkinson-like disease [10]. Related to that matter, various studies reported that *M. pruriens* contains L-dopa [11, 12], a dopamine precursor, that can infiltrate into the blood-brain barrier, thus it helps to recuperate the dopamine levels [13]. Sardjono et al. [14] explained that specifically, *M. pruriens* seed from Indonesia contains 7.56 to 13.9% of L-dopa, in addition to other contents such as alkaloids, steroids, saponins and tannins. Therefore, it is not surprising many studies stated *M. pruriens* seeds as a potential antiparkinson [15-17]. To evaluate its effectiveness in treating Parkinson's disease, an animal model of Parkinson's disease is usually used. Administration of haloperidol in animals will cause catalepsy, a condition characterized by muscle stiffness, decreased sensitivity to the stimuli even painful stimuli and a tendency to perpetuate an immobile posture [18, 19] as seen in people with Parkinson's. Study related to the activity of *M. pruriens* seed extract showed that the administration of the extract at the dosage of 200 and 400 mg/kg bw lowered the catalepsy in mice significantly [12].

Currently, the rapid development of nanoparticle technology makes it widely applied in many areas including the biomedical field. Nanoparticle drugs are stated to have advantages over conventional medicine. It is known that the change of the drug sizes to be a nanoparticle ameliorates its compatibility and bioavailability. It can be suspended in a liquid without difficulty and able to penetrate the organs and tissues. According to Poupot et al. [20], nanoparticle drugs provide a better impact when used in the treatment of several diseases, including Parkinson's disease. Since nanoparticle drugs enhance advantages over the conventional drugs because of their properties, synthesis of nanoparticles is now widely favoured. Magnetite nanoparticle has attracted interest because of its properties such as can form ultrafine size, is superparamagnetic and high biocompatibility [21, 22]. Magnetite nanoparticle has been used in magnetic resonance imaging (MRI), drug delivery systems, gene therapy, early detection of inflammation, imaging of cancer, atherosclerosis, diabetes and others [23, 24]. In clinical applications, magnetite

nanoparticles larger than 200 nm in size can be filtered by the spleen while smaller than 10 nm in size can be excreted through renal clearance. Therefore, magnetite nanoparticles are expected to have a size between 10 and 200 nm [25].

Nanoparticles are usually synthesized by various physical and chemical methods. Merely, for gaining the greener process, nowadays the biological material such as microorganisms and plant extracts are employed for the bioreduction of metal salts during the nanoparticle synthesis. This method is considered simple, cost-effective, non-toxic, energy-efficient and more environmentally friendly. Furthermore, employing the plant extract in the synthesis of nanoparticles is easier, faster and cheaper compared to microorganisms [21, 22, 26]. Various studies have been synthesized magnetite nanoparticles by using plant extract resulting magnetite nanoparticles in a variety of shapes and sizes [21, 27-29].

Studies by Rahmani-Nezhad et al. [30] and Teimuri-Mofrad et al. [31] showed that *M. pruriens* seed extract can be used in the biosynthesis of metal nanoparticles with a rapid process at room temperature and produced stable nanoparticles. The previous study reported that motor system abnormality in animals caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) could be repaired significantly by the administeration of the gold nanoparticle from *M. pruriens* seed extract at the dosage of 10 mg/kg bw compared to the administration of *M. pruriens* seed extract itself [32]. These results suggest that the extract in nanoparticle size provide better bioactivity.

The research facts have shown the potency of *M. pruriens* in developing of magnetite nanoparticle as one of the promising medicine in lowering catalepsy. However, to date, no studies have been reported on the synthesis of magnetite nanoparticles using *M. pruriens* seed extract from Indonesia as its reducing agent. Thus, our work aimed to synthesize and characterize the magnetite-*M. pruriens* seed extract nanoparticles (FeMPn) from Indonesia as well as evaluate its bioactivity to catalepsy as a part of the development of herbal medicine for Parkinson disease treatments.

2. Experimental Procedure

2.1. Materials

The seeds of *M. pruriens* were acquired from Bantul, Yogyakarta. The botanical identity of the plant specimen was confirmed at School of Life Sciences, Institut Teknologi Bandung, Indonesia, with voucher specimen 468/11.CO2.2/PL/2017. Ethanol 96% (technical grade), citric acid (p.a), $FeCl_3$ (p.a), L-dopa, haloperidol, PGA (poly-glutamic acid) and animal feed (CP511) were obtained from local (Bandung) suppliers.

2.2. General procedure for the preparation of M. pruriens seed extract

The sun-dried *M. pruriens* seeds ground to a fine powder and obtained a total of 5.9 kg of seed powder. The seed powder was extracted by maceration using water-ethanol (1:1) at room temperature. Maceration was conducted three times, each for 24 h with daily solvent replacement (3×24 h). Citric acid was added to obtain *p*H 3 during maceration. Macerate then filtered to obtain 12 L of the liquid extract. The solvent in the liquid extract was evaporated at 40 °C under low pressure in a rotary vacuum evaporator while the water content was removed by using a frozen dryer to obtain 242.70 g (4.1%) of the dry extract [12, 33].

2.3. Synthesis of FeMPn

The 50,000-ppm extract solution was prepared by dissolving the 5 g of *M. pruriens* seed extract into 100 mL of distilled water then homogenized with magnetic stirring for 15 min. To synthesize FeMPn, the extract solution was added to 0.08 M $FeCl_3$ solution dropwise with a volume ratio of 1:1. To homogenize the reaction mixture, sonication was deployed for 20 min then ultrasonic homogenizer for 20 min, after that the mixture was kept at room temperature for 24 h. To separate the solid from its liquid, the suspension formed was centrifuged at 10,000 rpm. FeMPn powder was obtained after the solid formed filtered and washed several times using distilled water. The black FeMPn powder was obtained after the solid dried in an oven at room temperature [21, 34].

2.4. Characterization

2.4.1. Scanning electron microscopy with energy dispersive X-ray spectrometry (SEM-EDX)

Characterization using SEM-EDX was performed to ascertain the morphology, size and composition of elements contained in FeMPn. SEM and EDX for elemental analysis were executed by using Hitachi SU3500 with coating ion sputter Hitachi MC1000 and Au as coater of the samples.

2.4.2. Transmission Electron Microscopy (TEM)

TEM was employed in imaging the nanoparticles produced to obtain the character of particle size, size distribution and morphology of FeMPn. TEM analysis was carried out using Hitachi HT7700.

2.4.3. Fourier-transform infrared spectroscopy

The compounds and functional groups contained in both *M. pruriens* seed extract and FeMPn were confirmed by analysing the Fourier transform infrared (FTIR) spectra. FTIR spectra were obtained from measurements using Shimadzu 8400 in the range of 4000-400 cm^{-1} wavenumbers. Samples were prepared in KBr pellets. The thermal stability of FeMPn was examined by TGA/DTA where nitrogen was used and the sample heated at a constant rate with 1-10 °C/min heating rate.

2.5. Experimental animals

12-weeks-old healthy male mice with 18-35 g bw were used in catalepsy test. Mice were maintained in polypropylene cages in a room at a temperature of ± 22 °C and adapted for a week before any procedures are performed. Mice were given a standard diet (CP511) and water. Permission to use animals in this study was issued by Universitas Garut.

2.6. Catalepsy test

The 27 mice were arbitrarily assigned to nine groups with three mice per group. The mice were given PGA 1%, L-dopa, the extract, or FeMPn at the respective dosage orally as per in Table 1. After 30 min of that, the mice were given haloperidol at a dosage at 5 mg/kg bw orally to induce the catalepsy. The catalepsy test was performed 30 min after the administration of haloperidol. Catalepsy was

measured by the bar method where the mice are placed in a position with its front legs holding on a bar suspended above the floor at 50 cm in height and 0.5 cm in diameter as described by [33]. The intensity of catalepsy is reflected from the length of time the mice maintain this position. Mice have suffered catalepsy if they hang on the wire without any moving for more than 15 seconds [7, 35].

Table 1. Catalepsy test groups.

Group	Treatment
Normal	Water
Negative control	Vehicle (PGA 1%)
Positive control	L-dopa 10 mg/kg bw
Extract	*M. pruriens* seed extract 200 mg/kg bw
Very low dosage	FeMPn 5 mg/kg bw
Low dosage	FeMPn 10 mg/kg bw
Moderate dosage	FeMPn 15 mg/kg bw
High dosage	FeMPn 20 mg/kg bw
Very high dosage	FeMPn 25 mg/kg bw

2.7. Statistical analysis

The statistical analysis of the data resulted from the catalepsy test was conducted to define the significant differences in the treatment given between the control and treated groups. SPSS 22.0 software was employed to run the one-way Analysis Of Variance (ANOVA) followed by Dunnett's multiple comparison tests. The significant differences were based on the P-value where $P < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Biosynthesis and characterization of FeMPn

The reaction mixture of the *M. pruriens* seed extract and ferric (III) chloride undergoes sonication for 40 min at room temperature. As the reaction proceeded, it was observed that the colour of the solution changed from brown to intense black, indicating the formation of magnetite nanoparticles. The formation of iron nanoparticles was indicated by a change in colour of reaction, medium to dark brown or even black. The change in colour into black also could be the signs of aggregation [21, 36].

The SEM images in Fig. 1 showed that FeMPn has a non-homogeneous and uneven surface, as well as the size of FeMPn that tended to vary due to aggregation. However, based on the TEM image in Fig. 2, FeMPn has spherical in shape with the smallest particle of 30.5 nm. This shows that the FeMPn produced meets the criteria for nanoparticle where the size is between 1-100 nm [21]. A non-homogeneous of FeMPn produced might be due to the lack of the reaction time or the stirring time thus impact the formation of nucleation imperfectly [37]. However, the size of FeMPn produced was within the range of magnetite nanoparticle size expected [25].

The result of EDX as in Fig. 3 showed that the Fe composition contained in FeMPn was 10.6%, while the other elements contained in FeMPn were carbon (40.4%), oxygen (33.0%) and nitrogen (10.5%), which possibly come from the compounds contained in *M. pruriens* seed extract that interact to the surface of

magnetite nanoparticles. Nevertheless, the EDX confirmed that the Fe ions have been reduced to Fe metals.

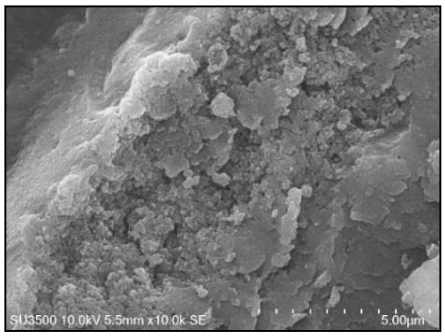

Fig. 1. SEM image of FeMPn.

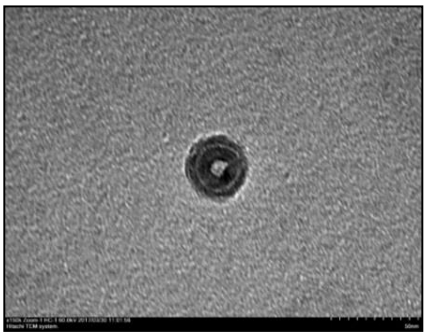

Fig. 2. TEM image of FeMPn.

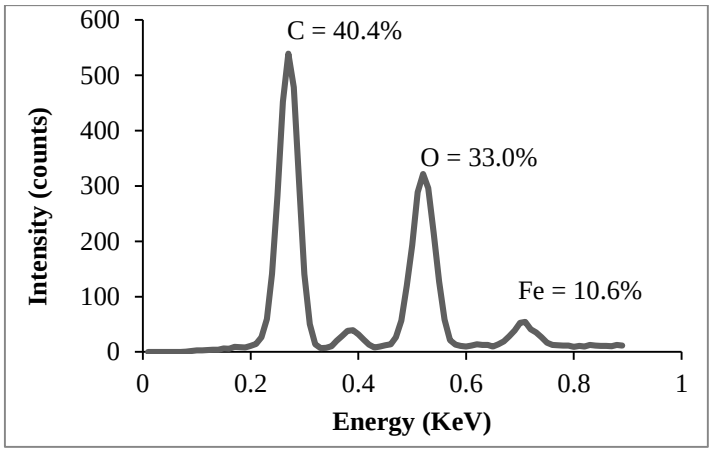

Fig. 3. EDX graphs of FeMPn.

Both *M. pruriens* seed extract and FeMPn were characterized by FTIR to ascertain the compounds contained in the extract that engaged in the reaction forming FeMPn. Figure 4 shows the FTIR spectra of the extract and FeMPn. The FTIR spectrum of the extract shows a broad absorption band in the absorbance area of 3384.8 cm^{-1} that assigned to the overlapping of *O-H* stretching vibration and N-H stretching vibration of amine compounds because of hydrogen bonding. This hydroxyl group is might be derived from flavonoids, alkaloids, polyphenols, alcohols or water. L-dopa that contained in the extract is an amino acid, which contains an aromatic ring and amine group besides hydroxyl and carboxyl groups. The *C = C* stretching vibration of the aromatic ring is denoted by the absorption band in the absorbance area of 1627.8 cm^{-1}, while the N-H bending vibration of amine is denoted by the absorption band at 1529.4 cm^{-1}. The hydrogen attached to sp^2 carbon of amino acids is proven by the emergence of an intense absorption band at 1400.2 cm^{-1} that referred as *C-H* bending vibration. The weaker absorption band at 1288.4 cm^{-1} corresponded to = *C-O* stretching vibration of aromatic compounds and the absorption band at 1074.3-1118.6 cm^{-1} referred to *C-O* stretching vibration of amino acid.

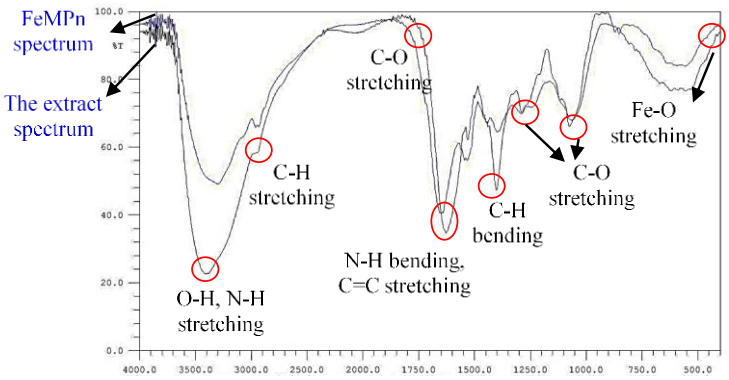

Fig. 4. FTIR spectra of the *M. pruriens* seed extract and FeMPn.

In the FeMPn FTIR spectrum, there are several changes in the intensity of the absorption band and the shifts in the absorbance area. These findings indicate the compounds contained in the extract interact in the formation of FeMPn. The emergence of *N-H, O-H, C=C* and *C-O* absorption bands refer to the presence of amino acids, specifically L-dopa, which may act as a reduction agent in the synthesis of FeMPn. L-DOPA has been reported as a reducing agent in the synthesis of nanoparticles. The functional groups in L-DOPA are able to reduce metal ions, thereby leading to the formation of nanoparticles. Besides reducing the metal ions, L-DOPA also leads to stabilize the nanoparticles formed [38, 39]. L-dopa reduced the Fe ions to form Fe metal while it oxidized to form dopaquinone [40]. It indicated by the decrease in the intensity of the absorption band in FTIR spectrum of FeMPn especially the absorption band at the absorbance area of 3384.8 cm^{-1} indicating that hydroxyl group has been oxidized to ketone as seen in dopaquinone. FTIR spectrum of FeMPn exhibited a typical absorption band at 557.4 cm^{-1}, which referred to the Fe-O interaction on FeMPn [41, 42].

From the FTIR of the extract and FeMPn analyses, it can be concluded that some of the biomolecules contained in the extract such as alkaloids, polyphenols, amino acids, especially L-dopa, were responsible for the biosynthesis of FeMPn and its stabilization. As reported, flavonoids, polyphenols, alkaloids, terpenoids, reducing sugars, nitrogenous bases and amino acids are reported to be responsible for the reduction of metal ions [22, 28, 29]. As a prediction, $FeCl_3$ was hydrolysed and formed $Fe(OH)_3$ then released H^+ ions. The acidic medium made the carboxylic acid such as in L-dopa being deprotonated. The deprotonated L-dopa reacted with Fe^{3+} ions produce dopaquinone and reduce Fe^{3+} ions into Fe^{2+} ions. These ions could react with the hydroxyl group to produce magnetite (Fe_3O_4), while dopaquinone stabilizes and protects the magnetite from oxidation during and after the synthesis [21, 43].

Figures 5 and 6 show the TGA/DTA profiles of the extract and FeMPn. The mass reduction at 140 ºC and an endothermic peak at 123.8 ºC on the TGA/DTA curve of the extract, while on FeMPn the mass reduction observed under 100 ºC and an endothermic peak emerged at 96.17 ºC. Studies by Ziegler-Borowska et al. [44] reported that the endothermic process can be explained as the breaking of hydrogen bonds followed by water evaporation, which leads to mass reduction. The

mass reduction and endothermic peaks also emerged at 200-300 ºC and above 300 ºC on the TGA/DTA curve of the extract and FeMPn respectively indicated the evaporation of some organic molecules. The broad exothermic peak at 280 ºC on the TGA/DTA curve of FeMPn indicated the bond breaking of L-dopa with magnetite nanoparticles. At a temperature of 550 ºC, the total mass of the extract remained was 38.37%, while it was 48.70% for FeMPn. From these results, it could be concluded that the addition of Fe to the extract impacted on the thermal stability that made FeMPn has better thermal stability than the extract itself.

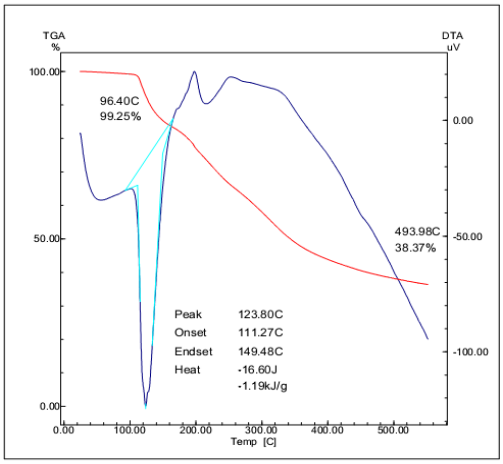

Fig. 5. TGA/DTA profile of the *M. pruriens* seed extract.

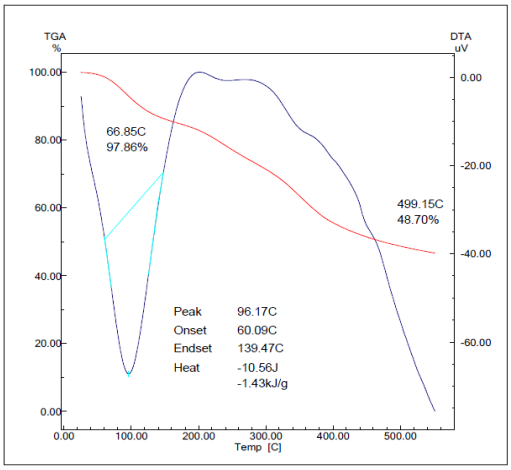

Fig. 6. TGA/DTA profile of FeMPn.

3.2. Catalepsy test

The catalepsy test was done by the bar method and accomplished 60 min after giving haloperidol and L-dopa, the extractor FeMPn per oral. In bar method, if the mice maintain the hanging posters with their front legs hold a wire with 0.5 cm in diameter and suspended above the floor at 15 cm in height without any moving for more than 15 seconds, thus they suffered from catalepsy. Figure 7 shows the data

of the catalepsy test. The mice of the negative control group maintain the hanging postures for more than 15 seconds, reflecting that the mice were suffered catalepsy. In contrast, the positive control, the extract and the FeMPn groups on the all five dosages have the hanging time less than 15 seconds. This finding indicated that the mice in the positive control, the extract and the FeMPn groups on the all five dosages were not catalepsy. However, their hanging time was still longer than the normal control group, which was 2 seconds. This finding demonstrated that catalepsy can be reduced by the treatment of L-dopa, the extract and the FeMPn on the all five dosages but the effect could not bring the mice back to normal. On the other hand, the hanging time of the FeMPn groups on the all five dosages was shorter than of the positive and the extract groups, which indicated that FeMPn on all five dosages were able to lower catalepsy better than L-dopa and the extract.

The statistical test was done with one-way ANOVA followed by Dunnett's multiple comparison tests and 0.05 limit of significance (95% confidence level). The result of the statistical test is shown in Table 2. The significant difference was seen when the groups treated with FeMPn at all dosages compared to a negative control as shown by P-value of less than 0.05, indicating that the catalepsy was reduced significantly by the FeMPn at the all dosages. The groups treated with FeMPn at the all dosages also showed the P-value less than 0.05 compared to a positive control, indicating the effect of FeMPn at all dosages in lowering catalepsy was better than of L-dopa 10 mg/kg bw. However, only FeMPn at the dosages of 10 and 20 mg/kg bw that reduced the catalepsy until the mice back to normal, confirmed by the P-value, which was more than 0.05 compared to the normal group showed the groups were not significantly different with the normal group.

A significant difference is considered as the $P < 0.05$. A statistical test was conducted by one-way ANOVA followed by Dunnett's multiple comparison tests.

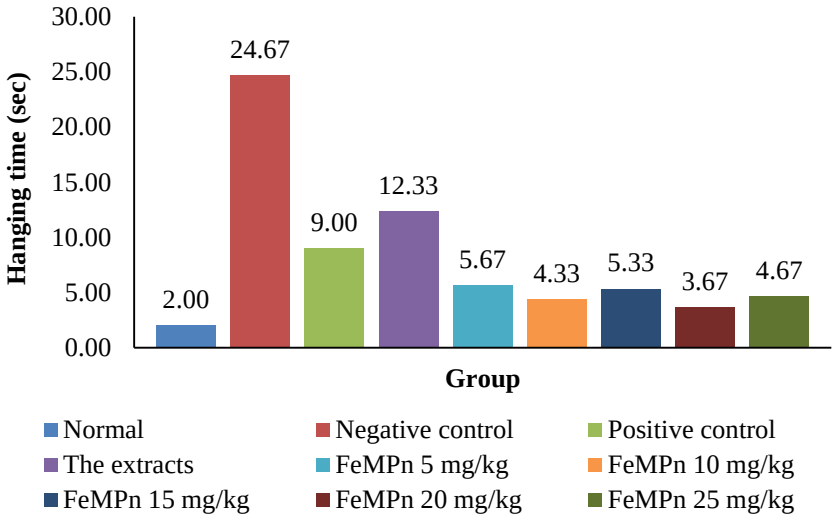

Fig. 7. Catalepsy test.

Table 2. The result of statistical test for FeMPn groups.

Groups treated with FeMPn	P-value compared to			
	Normal	Negative control	Positive control	The extract
5 kg/mg bw	0.016	6.0×10^{-6}	0.029	5.4×10^{-5}
10 kg/mg bw	0.151	2.4×10^{-6}	0.003	1.0×10^{-5}
15 kg/mg bw	0.029	4.7×10^{-6}	0.016	3.5×10^{-5}
20 kg/mg bw	0.398	1.6×10^{-6}	0.001	4.6×10^{-5}
25 kg/mg bw	0.088	3.0×10^{-6}	0.005	1.5×10^{-5}

This study also showed that nanoparticles increase the bioactivity of the extract itself. It was seen from the P-value of the groups treated by FeMPn at the all dosages, which is less than 0.05 compared to the group treated by the extract. This finding assured the activity of FeMPn in lowering catalepsy was significantly different compared to the extract. From these data, we can conclude that FeMPn could reduce the catalepsy better than the extract even in the lower dosage (10 mg/kg bw). This finding also in line with the previous report [32], which concluded the nanoparticle has better bioactivity than the extract itself.

4. Conclusion

Biosynthesis of magnetite-Indonesian velvet beans (*M. pruriens*) nanoparticle has been successfully carried out by reacting $FeCl_3$ solution with *M. pruriens* seed extract. The results showed that FeMPn formed aggregation with a spherical in shape and the smallest particle was 30.5 nm. The absorption band at 557.4 cm^{-1} on FTIR spectra indicated the existence of Fe-O interaction from FeMPn. The FeMPn has better thermal stability than the extract. FeMPn at a dosage of 5, 10, 15, 20 and 25 mg/kg bw could lower the catalepsy in mice significantly. The activity of FeMPn in lowering catalepsy was better than *L*-dopa 10 mg/kg bw and *M. pruriens* seed extract. The best dosage of FeMPn in lowering the catalepsy was 10 mg/kg bw. Future work needs to evaluate the safety of the FeMPn as the potential drug for Parkinson's.

Abbreviations

ANOVA	One-way analysis of variance
DTA	Differential thermal analysis
EDX	Energy dispersive X-ray
FeMPn	Magnetite-*M. pruriens* seed extract nanoparticles
FTIR	Fourier-transform infrared
PGA	Polyglutamic acid
SEM	Scanning electron microscope
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis

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