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Synthesis and antiparkinsonian activity of nanocomposite of chitosan-tripolyphosphate-*Mucuna pruriens* L extract (CS-TPP-MP)

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Abstract. *Mucuna pruriens* L. (MP) has antiparkinsonian activity because it contains levodopa that acts as a dopamine precursor and plays a role to stimulate dopaminergic receptors in Parkinson's sufferers. The therapeutic efficacy of MP extract can be improved by using a nano drug carrier system, such as chitosan-tripolyphosphate (CS-TPP). This study aims to synthesize, characterize, and evaluate antiparkinsonian activity of chitosan-tripolyphosphate-MP extract (CS-TPP-MP) nanocomposite in mice. MP seed powder was extracted by maceration method using water-ethanol (1:1) by adding citric acid until it reached pH 3. The CS-TPP-MP nanocomposite was synthesized by using ionic gelation method with variations in reactant composition and reaction time. The CS-TPP-MP nanocomposite was characterized by Scanning Electron Microscopy-Energy Dispersive X-ray (SEM-EDX), X-Ray Diffraction (XRD) and Fourier-Transform Infrared (FTIR) Spectroscopy. Catalepsy test was performed to find the antiparkinsonian activity level of CS-TPP-MP nanocomposite at doses of 5, 10, 15, 20, and 25 mg/kg body weight. Based on the results of CS-TPP-MP synthesis, it was found that the reactant composition (CS-TPP:MP) of 1:3 with reaction time of 20 minutes produced the highest yield (14.21%). SEM-EDX characterization showed that the morphology of CS-TPP-MP nanocomposite was predominantly spherical and the size was approximately 120-170 nm with a composition of C = 55.43%, O = 30.46%, N = 13.46%, P = 0.44%. XRD diffractogram showed that CS-TPP-MP nanocomposite has amorphous structure. FTIR analysis showed the appearance of absorption at wavelength of 1643.35 cm⁻¹ which proved the interaction between the primary amine group of chitosan and the carbonyl group of EMP. Catalepsy test demonstrated that CS-TPP-MP nanocomposite at the doses of 5, 10, 15, 20, and 25 mg/kg body weight could reduce catalepsy symptoms in mice significantly, and the best dosage was 20 mg/kg body weight.

1. Introduction

Parkinson's disease is a common degenerative disorder of the central nervous system after Alzheimer's disease [1,2]. Parkinson's disease is due to the decrease of dopamine levels, therefore certain nerve cells in the brain break down and cause abnormal movement and emotion [3,4]. One of the main symptoms of Parkinson's disease is catalepsy. Catalepsy is a disorder of the central nervous system characterized by muscular rigidity and fixity of posture regardless of external stimuli, as well as decreased sensitivity to pain [5-8]. To reduce catalepsy, it is advantageous to use synthetic drug, such as carbidopa, and levodopa. However, the drugs have side effects of prolonged use, namely dyskinesia, digestive disorders, orthostatic hypotension, insomnia, liver abnormalities, visual



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hallucinations, and depression [9-12]. Therefore, it is necessary to discover herbal medicine with minimum side effect. The minimum side effect of herbal medicine is resulted from extensive activity of its chemical constituents and its product [13-18].

The velvet bean plant (*Mucuna pruriens L.*) has been used by the ancient Indians for the treatment of Parkinson's disease as it possesses approximately 7.56-13.9% levodopa [19,20]. Its extract is more effective and has lower toxicity than synthetic drugs [20].

The implementation of nanotechnology for herbal medicine delivery system is a recent approach. The strategy is more beneficial since herbal plant extract is presented in nanoparticle structure (1-200 nm) made from biodegradable and biocompatible polymers; it is chitosan aiming at increasing solubility, bioavailability, and pharmacological activities [21-25].

Chitosan is a natural polysaccharide which can mostly be identified in the exoskeleton Crustacea, insects, and fungus. The molecular structure of chitosan is a linear polysaccharide composed of polymer glucosamine (β -1,4-2-amino-2-deoxy-D-glucose) [26,27,28]. Chitosan, in medical treatment, can be implemented in the regeneration of epithelial tissue, bone and teeth, wound dressing, antimicrobial, antioxidant, and anti-inflammatory. In addition, chitosan can also be considered to be a nanocarrier for some drug delivery systems. Furthermore, there are also some supportive data on biocompatibility of chitosan related to its function in nanomedicine [26,29]. For the specific reason, it is necessary to synthesize the chitosan-tripolyphosphate and MP extract (CS-TPP-MP) nanocomposite. The result will be beneficial as a drug for Parkinson's disease, specifically to reduce the symptoms of catalepsy in *Mus musculus*

2. Experimental section.

2.1 The Synthesis of CS-TPP-MP Nanocomposite

CS-TPP-MP nanoparticles were synthesized by employing the modified ionic gelation method [30]. In summary, 16 mL of tripolyphosphate solution (TPP) 0.07% (w/v) was added successively into 15 mL of chitosan solution (CS) 0.2% (w/v) then homogenized by magnetic stirrer. MP seed extract (10,000 ppm) was added successively into CS-TPP. Each solution was processed to sonication for 30 minutes, both before and after mixing process. The optimization of synthesizing CS-TPP-MP nanocomposite was performed on variations of CS-TPP and EMP reagent compositions of 1: 3, 1: 2, 1: 1, 2: 1, and 3: 1. It was also performed at some reaction time; 5, 10, 20, 40, and 60 minutes.

CS-TPP-MP solution was into shaker process for 12-15 hours at room temperature then centrifuged at 4000 ppm for 90 minutes. The formed residue was washed by aqua, moved on demineralization process, and dried at room temperature then, finally stored in desiccators. The synthesis results were identified by SEM-EDX (JSM-IT300 InTouchScope) to determine size, product morphology, and chemical composition of the product. To discover the functional groups, the analysis was performed by FTIR spectroscopy (Prestidge 21 Shimadzu). The X-ray diffraction patterns were obtained by using an X-ray diffractometer (Panalytical Xpert) operated at 40 kV and 30 mA with Cu-K α radiation ($k = 1.54060\text{\AA}$).

2.2 The Catalepsy Test

The catalepsy test was performed by a modified bar test method [31,32,33]. It was regarded with placing the animal test in hanging position on its two front legs, after neuroleptic treatment, namely haloperidol.

The animal test was male mice (*Mus musculus*) in age of three months with 23-38 grams as their weight. The mice were kept at standard condition $\pm 22^\circ\text{C}$ in polypropylene cage for approximately one week to adapt to laboratory condition. CP 551 and mineral water were fed into the mice. The mice were distributed into nine different groups. Each group consisted of three animals. The nine groups were categorized as normal, negative control, positive control, and experimental group (CS-TPP-MP dose at 5, 10, 15, 20, 25 mg/kg of body weight and MP extract 200 mg/kg of body weight).

The intensity of the catalepsy was measured as the length of time for the mice in hanging position on their two front legs holding a wire with 0.5 cm as its diameter and 8 cm as its height without doing any moves. The catalepsy observation was performed after giving haloperidol suspension 5 mg/kg of body weight at interval of 30-60 minutes. Haloperidol was transformed to the mice intramuscularly in 30 minutes after oral treatment of carrier suspension (PGA 1%), levodopa, MP extract or CS-TPP-MP at each predetermined dose.

The data was processed statistically using the One-Way Analysis of Variance (Anova) followed by Post Hoc Tukey test to evaluate significant differences between the groups. The data was analysed using SPSS 20 software and was considered significant at $P < 0,05$.

3. Result and discussion

The used extraction method was maceration since it does not require heating process during the extraction. Therefore, the thermolabile compound, as a major compound in MP seed, will never be damaged [34]. A research conducted by Misra and Wagner [35] shows that levodopa dissolves well in water-ethanol (1:1) which is acidified by the addition of citric acid to pH 3. The addition of citric acid aims at determining the pH. The use of ethanol aims at performing membrane cells denaturation, so that the extraction process of active compounds is more effective [36]. The dried extract obtained from the extraction of tangible black solid is 161.3 grams with the percentage of yield was 2.68%.

The Synthesis and Characterization of CS-TPP-MP Nanocomposite

The formation of CS-TPP-MP nanocomposite was performed by the change in color of CS-TPP solution, from colourless to deep black after being mixed by MP extract and put in sonication process. Ultrasonic waves (>20 kHz) can break down aggregates or agglomerates as well as polymer degradation through a process known as cavitation to produce small particles [37,38]. The adjustment of the parameters of reactant composition and reaction time is necessary to determine the optimum conditions based on the percentage of yields.

Variations in reactant composition, in fact, does not result a significant change to the percentage of yield. The reactant composition (CS-TPP: MP) 1:3 results the highest percentage compared to other reagent compositions. It has resulted product of 14.21%. This shows that more MP extract products are trapped in CS-TPP-MP nanocomposite.

The formation of nanocomposite which is an interaction between CS-TPP and MP extract, in fact, depends on the reaction time. The number of yields of CS-TPP-MP nanocomposite increases in the reaction time of 5 to 20 minutes, but at 20 to 60 minutes, the yields decreases. This confirms the previous experiment conducted by Buranachai *et al.* [39] stating that longer reaction can reduce the percentage of synthesis result as the swelling process is possible to release the filler that initially already entangled.

The result of SEM characterization (Fig 1) shows that the morphology of CS-TPP-MP nanocomposite is spherical with 120-170 nm in size. The respective size of CS-TPP-MP nanocomposite is at the criteria for nanoparticles; it is in the range of 100-200 nm [21]. Based on the EDX result in Fig 1, it is known that the CS-TPP-MP nanocomposite contains of elements C, O, N and P with the percentage of 55.43%; 30.46%; 13.46% and 0.44%. This shows the fixed formation of composite between chitosan, tripolyphosphate and MP extract. The existence of chitosan and MP extract is indicated by the presence of elements C, O, and N, while the existence of tripolyphosphate is indicated by the existence of element P. However, the data cannot be sure to confirm the composition of CS-TPP-MP nanocomposite.

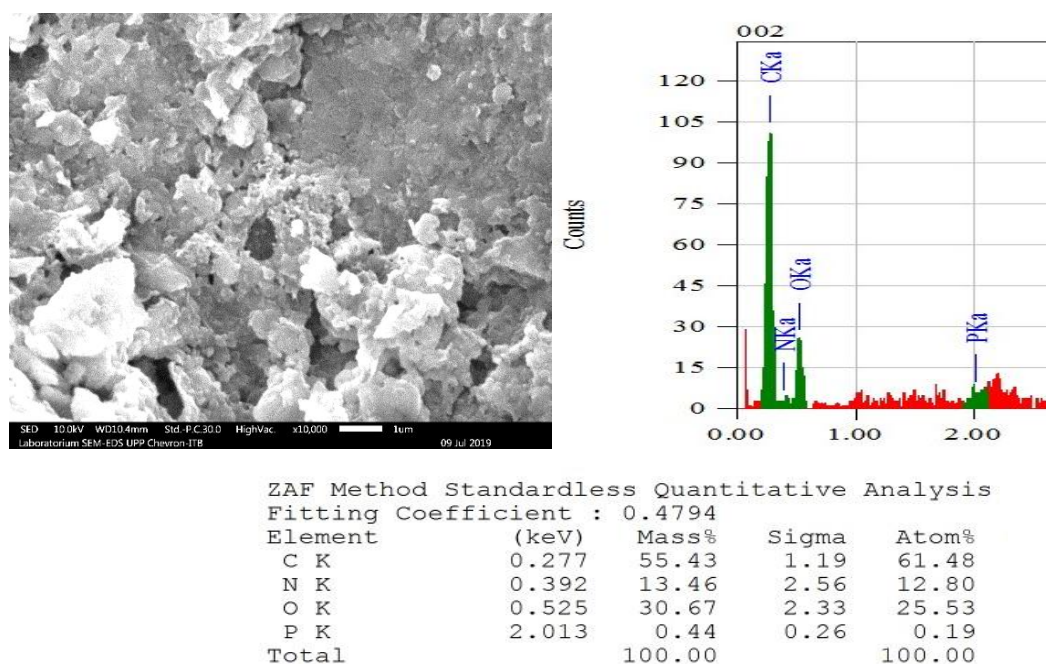


Figure 1. SEM visualization of CS-TPP-MP nanocomposite

The functional groups discovered in MP extract and CS-TPP-MP nanocomposite can be characterized by FTIR spectroscopy. Based on FTIR CS-TPP-MP, the typical *schiff base* exists at wave number $1632, 53 \text{ cm}^{-1}$ that shows the interaction between the primary amine group of chitosan and the carbonyl group of MP extract. FTIR CS-TPP and CS-TPP-MP spectra show the absorption group of $\text{P}=\text{O}$ at wave number 1150 cm^{-1} . The difference in intensity and shift in wave numbers are due to the decrease of hydrogen bonds. It shows that there is an interaction between MP extract and chitosan-tripolyphosphate [40].

The X-Ray Diffraction (XRD) was performed to identify the crystal structure of CS-TPP-MP nanocomposite. The diffractogram shows that the CS-TPP-MP nanocomposite possesses low-crystallinity and is amorphous (random and irregular pattern) so that the identified peak point of 2θ is different from literature, namely the absorption for CS-TPP is in the range of 20.20° , CS-TPP-LD is in the range of 8.06° , 16.04° and 24.52° , and the diffraction peak 2θ for the typical levodopa will exist at 16.04° [41,42]. In addition, the peak absorption of 2θ for CS-TPP-MP nanocomposite is at 54.10° and 69.38° . Amorphous crystal structure is resulted from the high composition of MP extract in CS-TPP-MP nanocomposite.

Catalepsy Test

The method was the bar method regarded with the observation of mice response when they are in hanging position on their two front legs holding a wire with 0.5 cm as its diameter and suspended 8 cm from the surface [31]. Mice are considered to experience catalepsy when they are in hanging position more than 15 minutes.

The negative control group (haloperidol treatment at 5 mg/kg of body weight) shows that the hanging time is more than 20 seconds. Therefore, the mice experience catalepsy. On the other hand, the positive control group (levodopa treatment dose at 10 mg/kg of body weight), MP extract 200 mg/kg of body weight, and CS-TPP-MP nanocomposite at 5, 10, 15, 20, 25 mg/kg of body weight show the hanging time is less than 20 seconds. It means that the mice do not experience catalepsy. Furthermore, the respective group has a longer hanging time than normal control group (1.55 ± 0.33 seconds). It shows that the positive control group (L-dopa treatment dose at 10 mg/kg of body weight), MP extract 200 mg/kg of body weight, and CS-TPP-MP nanocomposite 5, 10, 15, 20, 25 mg/kg of body weight are able to reduce the symptoms of catalepsy but have not yet reached normal condition. In addition, the hanging time for the group of mice with CS-TPP-MP nanocomposite doses of 5, 10, 15, 20, 25

mg/kg of body weight is smaller than the group of mice with MP extract. A prior research conducted by Sardjono *et al.* [43] shows the identical result. The antiparkinsonism activity of nanoparticles extract is more effective than MP extract. The hanging time of mice treated by CS-TPP-MP nanocomposite with dose at 20 and 25 mg/kg of body weight does not have significant change. It is possible as the optimum dose in mice is at 20 mg/kg of body weight.

The *one-way* ANOVA statistical analysis continued by *Post Hoc* Tukey test were performed to determine the significance of CS-TPP-MP nanocomposite in reducing catalepsy symptoms. The used significance limit is 0.05 with the confidence level of 95%. If the P value is less than 0.05, then the data show a significant difference. Based on the results of statistical tests, it shows that CS-TPP-MP doses of 5, 10, 15, 20, 25 mg/kg of body weight have a significance value of less than 0.05 to negative control groups. It shows that the hanging time of mice groups with CS-TPP-MP doses of 5, 10, 15, 20, 25 mg/kg of body weight has a significant difference to negative controls. This proves that CS-TPP-MP nanocomposite doses of 5, 10, 15, 20, 25 mg/kg of body weight can reduce the symptoms of catalepsy.

The result of comparison of the hanging time of mice groups with CS-TPP-MP nanocomposite doses of 5, 10, 15, 20, 25 mg/kg of body weight to normal control has a significance value less than 0.05. It indicates that the hanging time of mice groups with CS-TPP-MP nanocomposite doses of 5, 10, 15, 20, 25 mg/kg of body weight have a significant difference to normal controls. This proves that CS-TPP-MP nanocomposite doses of 5, 10, 15, 20, 25 mg/kg of body weight can reduce the symptoms of catalepsy but have not yet reached normal condition.

Conclusion

Based on the results of CS-TPP-MP nanocomposite synthesis, it can be concluded that the composition of CS-TPP: MP reagents is 1: 3 and the reaction time is for 20 minutes. It results the highest percentage of yields, 14.21%. CS-TPP-MP nanocomposite has spherical with 120-170 nm. The identified compositions are C=55, 43%, O=30, 46%, N=13,46%, and P=0,44%. The catalepsy test shows that CS-TPP-MP nanocomposite is able to significantly reduce the symptoms of catalepsy with the best dose of 20 mg/kg of body weight.

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