

Nano Calcium Resulted from *Anodonta Woodiana* as Mercury Detoxifying Supplement for Autism Children

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ABSTRACT

Small particle size of Nano Calcium from Freshwater Mussels shell will tend to increase the absorption of calcium by human's body and will be very well consumed by children with special needs to suppress hyperactivity disorders that caused by mercury. This study aims to create and determine the best supplement formulation containing nano calcium from Freshwater Mussels shell. The research method begins with the manufacture of Freshwater mussels shell powder and isolated nano calcium. Nano calcium is tested for its solubility in acid. The granule formula is made with stevia variations. Instant granule evaluation includes granule flow test, break angle test, compressibility test, water content test, solubility test, and hedonic test. The results show that nano calcium is soluble in 1:1 ratio of citric and tartrate acid, so that each formula needs 1,2% citric and 1,2% tartrate acid. Based on the flavor, aroma and texture, the most preferred formula contains 600 mg nano calcium, 60 mg citric acid, 60 mg tartaric acid, 400 mg stevia, 50 mg PVP and 3,83 g lactose. The best instant granule evaluation results show that the flow rate is 4,253 g / sec, 33,9^o rest angle, 4,65% compressibility, 2,21% water content and soluble time 58 second.

Keywords : Freshwater Mussels , Nano calcium, instant granule, detoxification, mercury

Introduction

Currently autism is a disease with a high indication (1: 250 children). Various factors may be the cause of autism, including metallothionein dysfunction and / or heavy metal poisoning. The detoxification of heavy metals and nutritional therapy with supplements containing zinc, cysteine and glutathione is recommended for the treatment of metallothionein dysfunction in autism (Santosa, 2003). Approximately 88 percent of autistic children have a damaged intestinal condition (autistic colistic). There is their suspicion of heavy metal poisoning. The process of removing toxins (detox) can be done by supplementation is divided into several categories, namely improve, provide essential nutrients, toxins, and improve neurotransmitters. One of the freshwater commodities that has the potential as a source of calcium is the shell of freshwater shells. Freshwater shellfish is one of the preferred freshwater commodities of the community. Utilization of freshwater shells as a source of Ca, Mg, P, Na, K, and Zn minerals help to overcome one of the problems in people with autism that is hyperactivity due to heavy metal contamination especially Hg. Particle nano powder from freshwater shells is one of the applicable uses in the manufacture of mineral supplements that can be used as a heavy metal detoxification supplement. With an increasing number of autistic people, such as a 56% increase in 1998 - 2013, supplement products containing nanocalcium powder have the potential to be developed as an alternative to a CFGF (casein free gluten free) diet and

its calcium content can help replace calcium from cow's milk Containing casein (Jeremy Y, 2015). Supplements containing nano calcium from freshwater shells can be consumed by autism children who are safe to eat and can suppress hyperactivity and can be consumed by people who have osteoporosis. Nanocalcium can reduce mercury contamination is also easily absorbed by the body. Utilization of freshwater shell shells as a supplement material can improve the usability and reduce waste so as to reduce environmental pollution. This study aims to create and determine the best supplement formula containing nano calcium from freshwater shells that can detoxify mercury based on hedonic test and quality test.

Materials and Methods

Tool

Equipment used include Hammer mill, hot plate, furnace, tray and basin, Filter 16, 20 and 30 mesh, Batik fabric, Oven, analytical balance, glassware, Granul flow tester Intralab brand, Tap density tester, Moisture balance brand AND.

Material

The materials used are freshwater shellfish (*Anodonta Woodiana*), HCl, NaOH, Citric Acid, Tartaric Acid, Stevia, PVP (Polyvinyl Piroolidon), Lactose, Essence, Dyes.

Method

The research stages include preparation of sample of gravestone, making of Taiwan Kijing shell flour, nanocalcium powder insulation, calcium nano solubility test, instant granule formulation making then hedonic test and quality analysis.

1. Making Flour from the shells of freshwater mussels

Samples of freshwater shells (*Anodonta Woodiana*) were obtained from the inundated waters of the Salabintana Experimental Pond Sukabumi. Freshwater shellfish used with a weight of about 200-300 g is cleaned and dumped. Shellfish flour is made by modification of the Sada method (1984), referred to in Wahyuni (2007) modified in the sieve stage. The shell of fresh water that has been separated from its dirt is cleaned, dried in the sun for 6-8 hours, then boiled in 1 N NaOH solution at 50°C for 3 hours. The boiled cinema is then neutralized by washing, then dried by oven At 121°C for 15 minutes. The dried freshwater mussels are then crushed using a hammer mill and filtered with a coarse mesh strainer and mesh mesh of 60 mesh to flour. Nano calcium is isolated from the resulting flour.

2. Isolation of nanocalcium from Shell flour of freshwater mussels

The flour was then extracted with HCl solvent at 90°C for 1.5 hours. The extraction results are further filtered by filter paper to obtain liquid / filtrate. The obtained filtrate was precipitated by addition of NaOH 3 N and stirring and sterilized until precipitation no longer formed. The precipitate obtained is then separated by decantation. The precipitate is then carried out a neutralization process using aquadest to pH 7. The next stage is the drying stage of sediment with oven and continued with combustion in the furnace at a temperature of 600°C so as to form calcium powder.

3. Nanocalcium Solubility Test and Instant Granule Formulation

The nanocalcium powder was tested for solubility in an acid solvent ie citric acid, tartaric acid and in 1:1 ratio of citric and tartrate acid. Use of amounts of citric acid and tartaric acid for pharmaceutical preparations 0.3-2.0%. Instant granule formulation of nanocalcium based on the best solubility test result and mercury detoxification test of previous research (Nanocalcium Dose 27 mg / 200 g body weight). The result of a child dose conversion for the use of calcium nano powder is 600 mg per day. The ingredients for the manufacture of instant granules such as nanocalcium powder, stevia, lactose, PVP are weighed at a certain amount, then sieved using a mesh screen 30. The nanocalcium, lactose, acid, stevia powder was put into a basin and then stirred until homogeneous for 5 minutes, added PVP that had been liquefied and left overnight then stirred until a compact mass. Instant granule formulation is made with variation of wet

stevia. Massa addition is sieved using mesh 16 sieve to form wet granule. Granul dried in a drying cupboard which has been covered by batik cloth at 40-50°C overnight to form dry granule. Dry granules were sieved using a mesh screen 20. In the manufacture of empty granules (placebo) for nanocalcium powder was replaced with lactose in equal amounts and in the same manner.

4. Evaluate Instant Granules

Instant granules that have been prepared according to formula with different use of stevia sweeteners are then evaluated. Evaluation of instant granules includes: granule flow test, break angle test, compressibility test, moisture test, solubility test and hedonic test.

a. Flow power test

The granular flow test is carried out with as much as 25 g of granules passed into the granule flow tester until the granule period passes through the funnel, then timed. Measurements were made 3 times.

b. Break Angel Test

Determination of the rest angle is done by inserting a mass of granules into the funnel. The falling mass will form a cone, then measured in height and diameter of the cone. This experiment was done 3 times.

c. Compressibility Test

The test is performed to determine the compressibility of granules and the attachment of binder with other granule materials. If the value of the compression increment before and after the tap is small, the more homogeneously mixed between the binder and the other granule. This test is done by weighing 50 g granules, inserted into the tube on the powder taping density tester. The granule volume in the tube is recorded before and after the stamping. This test was performed three times and calculated the price of compressibility (%) by the Carr equation (Aulthon, 1988).

d. Water content test

Examination of granule water content is done by using Moisture Balance. Each formula is inserted 1 g of instant granules into the prepared tool, at 105°C for 10 minutes and then the levels listed on the Moisture Balance are recorded

e. Solubility Test

A total of 8 g of instant granules is introduced into 250 ml water, then calculated by stopwatch, until the entire instant granules dissolve and record the time listed in the stopwatch.

f. Organoleptic Testing

Pengujian organoleptik yang dilakukan adalah uji mutu hedonik terhadap warna, aroma dan tekstur dan rasa dari granul yang telah dilarutkan. tests conducted on panelists in the age range 18-30 years as many as 20 people. Testing is done to find out the most preferred formula by panelists.

Results

Solubility Result of Nanocalcium and Instant Granule Formulation

Nanocalcium powder obtained from freshwater mussel flour has a white color. The yield of the obtained nanocalcium powder was 8.53%. The results of the solubility test showed that the nanocalcium soluble in a mixture of citric acid and tartaric acid 1: 1. Based on the results of the solubility test subsequently prepared an instant granule formula of nanocalcium. Instant granule formulas are made containing 1: 1 ratio of citric acid and tartaric acid taking into account that the use of citric acid and tartrate for pharmaceutical preparations in the range of 0.3-2.0%. The Instant Granule Nano Calcium Formulation is shown in Table 1. The prepared nanocalcium granules have a light yellow, granular, weak odor and slightly sweet to sweet taste. Evaluation done on physical properties of instant granules aims to ensure the quality of granules produced. The evaluation includes the nature of flowability, rest angle, compressibility, water content and solubility. The results of the evaluation of the instant nanocalcium granules are shown in Table 2.

Table 1. Formulation of Instant Granul Nano

Instant Granule Material	The amount added to the formula				
	FI	FII	FIII	Plasebo	
Nano kalsium	600 mg	600 mg	600 mg	600 mg	
Citric acid	6 mg	6 mg	6 mg	6 mg	
Tartaric acid	6 mg	6 mg	6 mg	6 mg	6 mg
Stevia	100 mg	300 mg	500 mg	300 mg	
PVP	50 mg	50 mg	50 mg	50 mg	
Laktose	4,2375 g	323 g	4,0375 g	323 g	4,0375 g
Aroma	qs	qs	qs	qs	
Dye	qs	qs	qs	qs	
Total	5 g	5 g	5 g	5 g	

Qs : quantum statis

Table 2. Results of Evaluation of Instant Granule

Test parameters	Instant Granule Formula			
	FI	FII	FIII	Plasebo
Flowability (g/sec)	3,417	3,787	4,253	5,297
Rest angle ($^{\circ}$)	34,76	32,37	33,90	29,99
Compressibility (%)	1,90	2,52	4,65	4,42
Water content (%)	2,057	2,183	2,21	0,76
Solubility (sec)	56,5	56,6	58	50

Discussion

Nano Calcium from Freshwater mussel shell flour

In this study, the method of precipitation is done by dissolving the calcium component mussels into the solvent acid (HCl) as the calcium dissolves in acid, then added a solution of NaOH to a solution of HCl that had been containing calcium. To mixing of the acid-base resulted in a saturated solution of calcium deposits and produce a smooth and nano-sized. According to Kenth (2009), precipitation method was done by the active substance dissolved in a solvent, then added another solution H_2CO_3 not solvent (anti-solvent), this leads to the solution became saturated and the nucleation occurs rapidly to form nano particles. Isolation of calcium from the local mussels carried by demineralization using HCl. This process is the process of dissolving minerals contained in mineral $CaCO_3$ mainly mussels. At the beginning of the mixing process Mussels with 1N HCl, formed a lot of froth and bubbles that lasted approximately ± 5 minutes. This is caused by the formation of the gases CO_2 and H_2O on the surface of the solution. Calcium content in the Mussels of calcium carbonate ($CaCO_3$) is reacted with HCl to form $CaCl_2$. Calcium chloride ($CaCl_2$) is formed and then performed with NaOH produces precipitation in the form of calcium hydroxide sludge and salt. Salt (NaCl) are formed during the neutralization process is eliminated, so that all that remains is $Ca(OH)_2$. Calcium hydroxide $Ca(OH)_2$ will be transformed into calcium oxide (CaO) during the process of incineration at a temperature of 600 $^{\circ}C$, as H_2O evaporates so that the end product is calcium oxide nano powder. The yield of the nanocalcium powder was obtained at the treatment time of 1.5 hours extraction was 8,53%.

Results of Instant Granul Evaluation

The results of instant granule evaluation of flow rate, rest angle and compressibility are presented in Table 2. Flow rate evaluation showed that the instant I granule I and II formulas had rates with cohesive characteristics whereas formula III and placebo flowed easily. Evaluation of the silent angle indicates that

all formulas have a good flow type that is easy to flow. Evaluation of the silent angle indicates that all formulas have a good flow type that is easy to flow, Because the instant granules of nanocalcium are more hygroscopic which gives rise to the adhesion-cohesion style, the surface area increases in the granules. Compressibility testing of all formulas of both nanocalcium granules and placebo granules has a compressibility index with an excellent flow type that is below 20%. Evaluation of granular moisture content showed that the nanocalcium instant granule formula met the requirements, the water content of formula I was 2.057%, the formula II was 2.183% and the formula III was 2.21%. The solubility test results showed faster soluble formulas I and II than formula III. The solubility of the formula I is 56.5 seconds, the formula II 56.6, 23 seconds and the formula III 58 seconds.

Organoleptic Granul Instant Test Result

Organoleptic tests were performed on instant granules after dissolving, and the tests were performed on aroma, color, taste and texture. The hedonic test was conducted by 20 panelists. The result of hedonic test in panel is concluded by using statistical analysis. The statistical results show that on the color parameter, all instant granule formulas show no difference. For taste, aroma and texture parameters of formula III are preferred granules compared to FI and FII.

Conclusion

Nano calcium from Freshwater Mussels shell(*Anodonta Woodiana*) is soluble in 1:1 ratio of citric and tartrate acid, so that each formula needs 1,2% citric and 1,2% tartrate acid. Based on the statistical results of the hedonic test on the instant granules showed that the color parameters were not significantly different, whereas the most preferred flavor, aroma and texture of the instant nanocalcium granule was formula 3. Instant granule formula 3 contains 600 mg nano calcium, 60 mg citric acid, 60 mg tartaric acid, 400 mg stevia, 50 mg PVP and 3,83 g lactose. The best instant granule evaluation results show that the flow rate is 4,253 g / sec, 33,9⁰ rest angle, 4,65% compressibility, 2,21% water content and soluble time 58 second.

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