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PUBLICATION TYPE	ISSN	COVERAGE	INFORMATION
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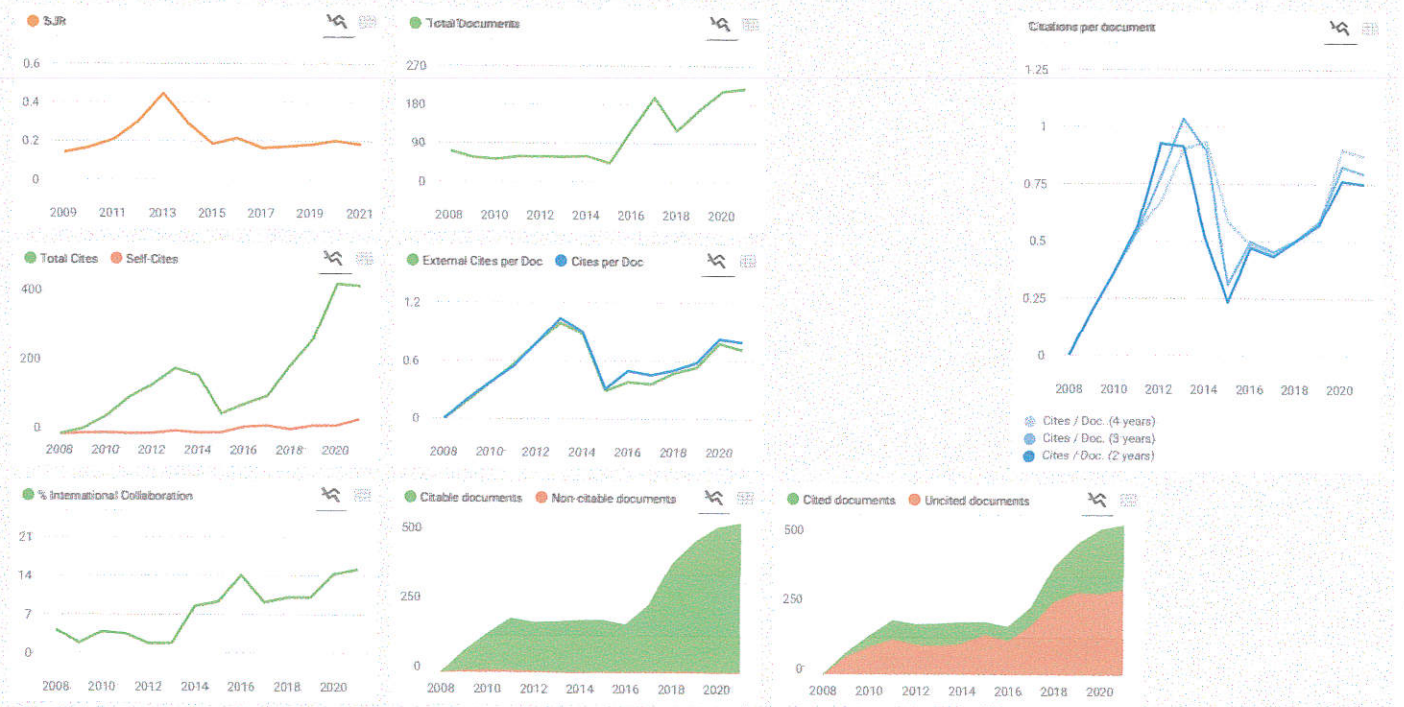
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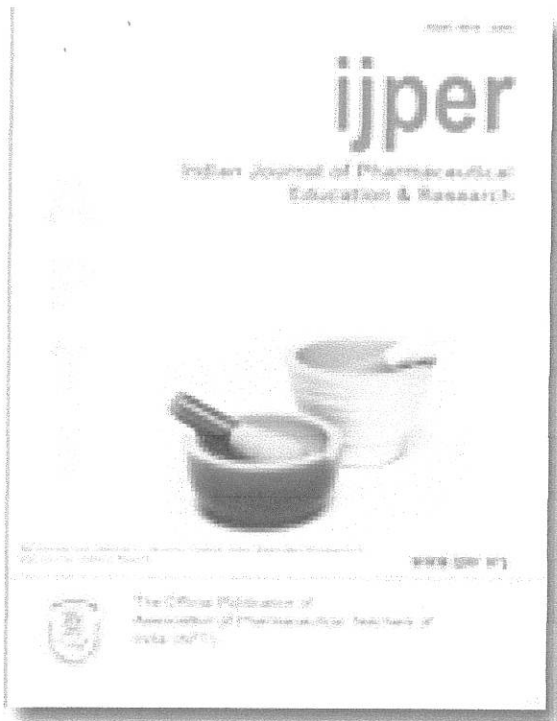
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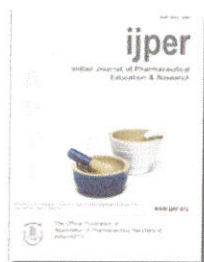
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Indian Journal of Pharmaceutical Education and Research

Indian Journal of Pharmaceutical Education and Research, Vol 55, Issue 3, Jul-Sep 2021



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 **Manuscript**



Oral Delivery of Purple Sweet Potato (*Ipomoea batatas* L.) Extract-Loaded Carboxymethyl Chitosan and Alginate Nanocapsule in Streptozotocin-induced Diabetic Mice

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ABSTRACT

Background: Insulin therapy is an essential part in diabetes mellitus type 2 treatment, but it has several side effects such as allergy and hypoglycemia. Therefore, alternative treatments are needed, one of which is by using nanocapsules to increase the performance of the drug delivery system. Purple sweet potato contain high anthocyanin levels which has antidiabetic properties. The idea of this research were to determine the potential use of purple sweet potato extract-loaded CMC-Alginate nanocapsules and to address problems regarding the efficiency of encapsulation to increase the bioavailability. **Methods:** This experimental research used post-test only control group design. The samples were 24 mice which were separated into four groups based on the administration of the following, respectively: 0.5 mL of extract-containing CMC-Alginate nanocapsules, 0.5 mL of extract, 1 mL of glibenclamide as the positive control and 0.5 mL of 0.5% CMC sodium placebo as the negative control. Mice were conditioned to be diabetic by induction of Streptozotocin. Blood glucose levels were carried out on days 1, 3, and 7 using an "Easy Touch" glucometer. The statistical analysis was conducted by two-way ANOVA proceeded by Tukey's *post hoc* test to investigate the differences between all groups. **Results:** Statistically, purple sweet potato extract-loaded CMC-Alginate nanocapsules, which its extract concentration was 4.4 times less than that of extract without encapsulation, can reduce high glucose levels in mice when as compared to negative control ($p < 0.05$). **Conclusion:** Purple sweet potato extract-loaded CMC-Alginate nanocapsules has capability to reduce blood glucose levels of streptozotocin-induced mice.

Key words: Alginate, Carboxymethyl Chitosan, Diabetes Mellitus, Nanocapsules, Purple Sweet Potato.

Key Messages: This research showed that the encapsulation of purple sweet potato extract with carboxymethyl chitosan-alginate has a capability to reduce high blood glucose levels in streptozotocin-induced diabetic mice and the encapsulation may enhance its efficiency, which exhibits great potential for further type 2 diabetes treatment.

INTRODUCTION

Type 2 Diabetes is a metabolic disease caused by the ineffectiveness of insulin hormone work due to abnormalities in insulin secretion such as by insulin resistance in the body or disruption of pancreatic β cells.¹ The pathogenesis of type 2 diabetes are caused by relative lacks of insulin, damage to pancreatic β cells due to environmental

influences, decreased glucose receptors in the pancreas, or damage to the insulin receptors in peripheral tissues. Continuous damage to pancreatic β cells can result in insulin deficiency that could force people with type 2 diabetes mellitus needing exogenous insulin.²

Submission Date: 08-12-2020;

Revision Date: 02-01-2021;

Accepted Date: 18-06-2021

DOI: 10.5530/ijper.55.3.143

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Insulin binds to the extracellular α subunit, resulting in a shape transformation in which ATP bonds occur in the intracellular component of β subunit. ATP bonds trigger phosphorylation through enzyme tyrosine kinase, also known as IRS. After passing through receptors, glucose is transported using glucose transporter (GLUT). GLUT-4 is the main transporter located in muscle and fat cells. Insulin resistance is caused by abnormalities in the function of GLUT-4, increased degradation of early insulin signaling molecules, or deficiency of phosphorylation of tyrosine insulin receptors, IRS protein, or PIP-3 kinase.³

Hyperglycemia is a common condition found in untreated diabetes patient. It can gradually lead to cause serious damage to organs, especially the nervous system and blood vessels.⁴ Insulin therapy is one of the most common treatments for people with diabetes in order to increase insulin levels in the blood and to reduce gluconeogenesis process by the liver. However, this therapy poses many side effects such as allergy and hypoglycemia. Increased insulin resistance in elderly patients can result in less effective insulin therapy.⁵

Purple sweet potato (*Ipomoea batatas* L.) pose high anthocyanin contents, ranging from 110 – 210 mg/ 100 gram.⁶ Anthocyanin are flavonoid phytopigments, water-soluble, and posing antioxidant properties.⁷ They have C6-C3-C6 structure and are positively charged. Purple sweet potato (PSP) contain anthocyanin which include cyanidin, peonidin, and pelargonidin.⁸ Previous research revealed that the antidiabetic mechanism of anthocyanin was performed by stimulating GLP-1 (glucagon-like peptide-1), a potent incretin hormone whose functions are to stimulate secretion and to trigger the proliferation of insulin hormone and pancreatic β cells for blood glucose levels.⁹

Nanocapsules are macromolecular substances or solid polymers with a size of 5 - 1000 nm. Chitosan, is a polysaccharide made up of (1-4)-linked 2-amino-2-deoxy- β -D-glucopyranose which is biodegradable, biocompatible, and is a mucoadhesive polymer that does not produce toxic and poses non-immunogenic properties. Therefore, it has advantages if administered orally as nanocapsules.¹⁰ The carboxymethyl chitosan (CMC) is a water soluble chitosan derivative.¹¹ The carboxymethyl chitosan and alginate nanocapsules are able to form polyelectrolyte complexed film, a biological membrane that has the effect of slowing and delaying the release of the core material contained in nanocapsules and maintaining structural stability of nanocapsules in the lumen of gastrointestinal tract.^{12,13} In addition, polyelectrolytes film also has affinity for the

intestinal mucosa, which can prolong the duration of residence time in the intestinal lumen, thereby increasing the bioavailability.¹⁴

Nanocapsules have broad potential and the advantages of slow or controlled drug release, thereby increasing drug's stability and solubility, increasing efficacy and reducing toxicity. Owing to their small size, nanocapsules can bypass biological barriers and head straight for their targets.¹⁵

Based on available literature and data, the idea of this research is to determine the potential use of PSP extract based on CMC-Alginate nanocapsules and to address problems regarding the efficiency of encapsulation using CMC-Alginate nanocapsules to increase the bioavailability of PSP extract.

MATERIALS AND METHODS

Materials

The extraction process of PSP was made with the maceration method using ethanol (96%) and tartaric acid (Merck) as solvents. All of these extraction processes were conducted at "Unit Layanan Pengujian" or Testing Service Unit of the Pharmacy Faculty of Airlangga University. The Carboxymethyl Chitosan and Alginate nanocapsules were purchased from Hainan Zhongxin Chemical Co., Ltd (No. 9012-76-4), Hainan, China.

Preparation of Nanocapsules

Purple sweet potato extract-loaded carboxymethyl chitosan and alginate nanocapsules were prepared according to a method that has been proposed previously by He et al. with slight modifications.¹⁶ Briefly, a total of 5.97 mg of purple sweet potato extract (\pm 12 mL extract) were added to 42.9 mg CMC solution (15 mL) followed by the drop-wise addition of 29.4 mg alginate solution (30 mL) into purple sweet potato extract-CMC mixture which was stirred at room temperature continuously for 30 minutes. Purple sweet potato extract-loaded CMC-Alginate nanocapsules suspensions that were mixed were then centrifuged at 15.000g for 20 minutes to separate the insoluble part of purple sweet potato extract-loaded CMC-Alginate nanocapsules (Figure 1).

Research Design

This study has been approved and granted exemption by "Health Research Ethics Committee of Medical Faculty of Airlangga University" (Approval No.291 / EC / KEPK / FKUA / 2020). The research was an experimental study that used experimental animals (*in vivo*) by administering PSP extract with "post-test

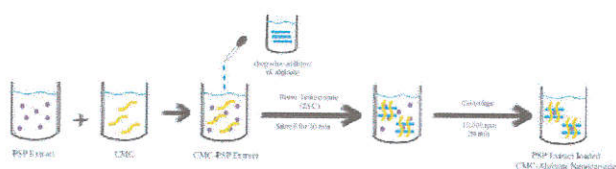


Figure 1: Encapsulation process of purple sweet potato extract with CMC-Alginate nanocapsules

only control group design” approach. The population was 24 mice, whose number was obtained from the Frederer formula, $[(t-1) (n-1)] \geq 15$ where t represents the number of treatments / number of groups and n represents the number of repetitions / size of the sample in the group.¹⁷ Mice (*Mus musculus*) which had been acclimatized to cage conditions for seven days were assigned into four different groups, each containing six mice. The following showed the division of the groups:

Group I :	Mice were given purple sweet potato extract based on CMC-Alginate nanocapsules at a dose of 0.5 cc during treatments.
Group II :	Mice were given only purple sweet potato extract at a dose of 0.5 cc during treatments.
Group III :	Positive control group, consisting of mice given glibenclamide 0.39 mg/L at a dose of 1 cc orally through force feeding needle during treatments.
Group IV :	Negative control group, consisting of mice given 0.5% CMC sodium placebo at a dose of 0.5 cc orally through force feeding needle during treatments.

Animal Models and Blood Examination

Adult male mice (*Mus musculus*) aged 12-14 weeks, with a weight ranging from 25 – 30 gram were used in this research. Mice were conditioned to be diabetic through induced by streptozotocin (STZ). Streptozotocin or 2-Deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose is produced by *Streptomyces achromogenes* and was used to induce type 1 or type 2 diabetes. Streptozotocin was freshly dissolved in citrate buffer at pH 4.5 and was given in single dose of 60mg/kg and was injected into mice intra-peritoneally. Insulin resistance was obtained by giving a high 10% dextrose diet.¹⁸ Examinations of blood glucose levels were carried out on days 1, 3 and 7 after induction of streptozotocin using an “Easy Touch” glucometer by taking peripheral blood samples from the lateral vein of the mice’s tail.

Statistical Analysis

The data obtained in this research were showed as mean ± standard error of mean (SEM), and evaluated by two-way ANOVA proceeded by Tukey’s post hoc test to determine the effect of differences in treatments

against the control group. The significance level used in this test was less than 0.05.

RESULTS

The blood glucose levels of mice that had been induced by STZ but not yet treated were measured and determined as day 0. Treatments were carried out for 7 days and measurements of blood glucose levels were carried out serially on days 1, 3 and 7. The results of the mean blood glucose levels of the four treatment groups are presented in Table 1.

The gradually decrease of blood glucose levels occurred in group I and group II. In group III, the positive control group, a very significant decrease occurred. In contrast, there was no significant decrease of blood glucose levels in group IV as a negative control group. The mean result of blood glucose levels on day 1 after treatment in the four treatment groups indicates significant difference ($p < 0.05$). The decrease of fasting blood glucose levels in group I and group II indicate insignificant differences in results compared to negative control group ($p > 0.05$), while group III showed a significant difference in results compared to negative control group ($p < 0.05$).

The result of blood glucose levels on third day of the treatment showed a significant difference between the four groups ($p < 0.05$). The decrease of blood glucose levels in group I, II and III showed a significant difference compared to negative control group ($p < 0.05$). The comparison between treatment group I and II found that there was insignificant difference in results ($p > 0.05$). The difference of the mean reduction in fasting blood glucose levels was ± 6.67 mg/dL.

On seventh day of the treatment, the mean results of blood glucose levels showed a significant difference

Group	Fasting blood glucose level (mg/dL)			
	0 day	1 st day	3 rd day	7 th day
I	148.33 ± 6.38	136.83 ± 7.46	127.17 ± 6.22*	131.83 ± 6.05*
II	182.83 ± 11.86	127.67 ± 4.65	120.50 ± 5.03*	110.33 ± 4.78*
III	181.33 ± 12.75	90.33 ± 13.62*	88.00 ± 9.07*	95.17 ± 3.94*
IV	175.67 ± 8.10	158.67 ± 11.99	165.00 ± 9.68	164.50 ± 8.18

Values are presented in mean ± standard error of mean (SEM) (n = 6 in each group). Group I: PSP Extract + CMC-Alginate nanocapsule; Group II: PSP Extract only; Group III: positive control (glibenclamide); Group IV: negative control (placebo), *p < 0.05 (compared to group IV as negative control).

between the four groups ($p < 0.05$). The decrease of blood glucose levels in group I, II and III showed a significant difference in results compared to negative control group ($p < 0.05$). On day 7, there was insignificant difference in results between treatment group I and II ($p > 0.05$).

DISCUSSION

This research aimed to analyze the potential of administering purple sweet potato extract based on carboxymethyl chitosan-alginate nanocapsules to decrease high levels of blood glucose in diabetic mice induced by streptozotocin. The results of this research showed that the anthocyanin level contained in PSP extract had a significant hypoglycemic effect to reduce fasting blood glucose levels.

Anthocyanin nature of being water-soluble and its effect of first-pass metabolism on the gastrointestinal tract reduce the bioavailability, making them less effective. CMC-Alginate nanocapsules are known to form polyelectrolyte films which increase the duration of anthocyanin in the digestive tract. Therefore, they can be used to overcome these problems and increase their bioavailability.¹⁹

After seven days of treatments, there was inconsiderable difference in the results of the mean blood glucose levels between the treatment group given PSP extract based on CMC-Alginate nanocapsules and the group given only PSP extract. These results prove that administering certain doses of PSP extract using the drug delivery method with nanocapsules can match the results of PSP at higher levels. This occurrence might be in connection with the formation of polyelectrolyte film so that the anthocyanin, contained in PSP extract, that stick to the lumen of the gastrointestinal tract can be released slowly.¹²

Treatment on the first day did not show a significant difference. This might be due to the dose of PSP extract given less than the initial dose. According to previous research conducted by Sutirta-Yasa and Jawi, the minimum anthocyanin from purple sweet potato extract dose required is 20.49 $\mu\text{g} / \text{mL}$ in diabetic mice. In previous research, it was stated that the peak levels of anthocyanin in the blood circulation were obtained after two hours of administration and that the anthocyanin levels were close to zero after 16 hr after administration.²⁰

In this research, group I was the treatment group given 0.5 mL anthocyanin encapsulate from purple sweet

potato based on CMC-Alginate nanocapsules with a ratio of 5.97 mg PSP extract : 42.9 mg CMC : 29.4 mg Alginate, similar to what had been done by He et al.¹⁶ Group II was the treatment group given 0.5 mL PSP extract. In this research, the researcher used a dosage volume of 0.5 mL because the maximum capacity of the mice's stomach was about ± 1 mL. The decrease of blood glucose levels in group I compared to negative control group proves that administration of PSP extract based on CMC-Alginate nanocapsules can reduce the blood glucose levels in mice ($p < 0.05$).

The effect of administration of PSP extract in group I and group II were seen on the significantly mean decrease of blood glucose compared to negative control group ($p < 0.05$). After the Tukey's test between group I and group II, it was found inconsiderable difference ($p > 0.05$). This showed that there was no significant difference between giving PSP extract based on CMC-Alginate nanocapsules and PSP extract only.

PSP extract encapsulate based on CMC-Alginate in group I contained extract levels of 0.103 mg / mL, equivalent to 0.0515 mg of PSP extract in one administration, while PSP extract in group II had extract levels of 0.466 mg / mL, equivalent to 0.233 mg of PSP extract in one administration. The comparison of PSP extract levels in the two groups can be described by the following equation:

$$\begin{aligned} & \frac{\text{Levels of PSP extract loaded in}}{\text{CMC-Alginate nanocapsules}} \\ &= \frac{\text{Levels of PSP extract in treatment group II}}{\text{Levels of PSP extract in treatment group I}} \\ &= \frac{0.0515 \text{ mg}}{0.233 \text{ mg}} \approx \frac{1}{4.4} \end{aligned}$$

In this case, with different levels of PSP extract given in group I and group II, both treatment group can lower blood glucose levels in mice. In fact, the levels of purple sweet potato extract contained in the CMC-alginate nanocapsules were 1/4.4 times the levels of anthocyanin in the treatment group II.

In conclusion, the result showed that the purple sweet potato extract-loaded CMC-Alginate nanocapsules was able to reduce blood glucose levels of diabetic mice induced by streptozotocin with 4.4 times greater efficiency compared to that of extract without encapsulation. Although encapsulation using CMC-Alginate nanocapsules could increase the bioavailability, this research still has some limitations for which the optimum dose of the encapsulation, its mechanism on blood glucose

reduction and its side effect on long term usage should be analyzed and studied further.

ACKNOWLEDGEMENT

The authors would like to appreciate the consideration of *Kementerian Pendidikan dan Kebudayaan Indonesia for the financial support, and Department of Pharmacology and Biochemistry of Airlangga University for providing the laboratory for this research.*

CONFLICT OF INTEREST

The authors declare no Conflict of interest.

ABBREVIATIONS

CMC: Carboxymethyl Chitosan; **GLUT:** glucose transporter; **PSP:** Purple sweet potato; **GLP-1:** glucagon-like peptide-1; **STZ:** Streptozotocin; **SEM:** Standard Error of Mean.

AUTHOR CONTRIBUTIONS

The authors contributed equally to this work. R.I., I.A.W., D.S.B., and N.R.P. designed this study; I.A.W., D.S.B., and N.R.P. performed experimental work; I.A.W., D.S.B., and N.R.P. provided original draft preparation; R.I. manuscript validation and supervision.

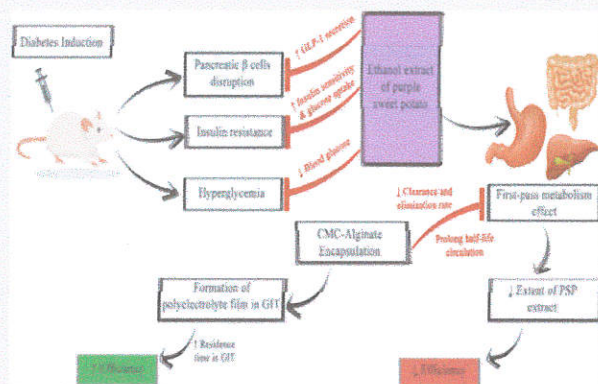
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SUMMARY

The anthocyanin contained in purple sweet potato had been proven to have antidiabetic ability by stimulating GLP-1, an incretin whose functions are to stimulate secretion and to trigger the proliferation of insulin hormone and pancreatic β cells, in order to reduce blood glucose levels. However, the first-pass metabolism that happens in gastrointestinal tract could possibly reduce the bioavailability and plasma concentration. The carboxymethyl chitosan-alginate nanocapsules are able to form polyelectrolyte complexed film that has the effect of slowing and delaying the release of the core material contained in nanocapsules and maintaining structural stability in the lumen of gastrointestinal tract. In addition, polyelectrolytes film also has affinity for the intestinal mucosa, which can prolong the duration of residence time in the intestinal lumen, thereby increasing the bioavailability. This study showed the potential of PSP extract loaded in CMC-Alginate through a statistical analysis that it can reduce blood glucose level of diabetic mice induced by streptozotocin with 4.4 times greater efficiency compared to that of extract without encapsulation. It indicates that the CMC-Alginate have an enhancing activity on the anthocyanin work, which exhibits great potential for further type 2 diabetes treatment.

PICTORIAL ABSTRACT



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Cite this article: I'tishom R, Wafa IA, Budi DS, Pratama NR. Oral Delivery of Purple Sweet Potato (*Ipomoea batatas* L.) Extract-Loaded Carboxymethyl Chitosan and Alginate Nanocapsule in Streptozotocin-induced Diabetic Mice. *Indian J of Pharmaceutical Education and Research*. 2021;55(3):709-14.