**Scholars Academic Journal of Biosciences**
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

**Biology**

# Utilization of Littopenaeus Vannamei Shrimp Shell Extract as a Blood Sugar Reducing Alternative

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**DOI:** 10.36347/sajb.2021.v09i07.002 | **Received:** 04.06.2021 | **Accepted:** 06.07.2021 | **Published:** 11.07.2021

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**Abstract** **Original Research Article**

Astaxanthin is a carotenoid pigment commonly found in a marine organism such as algae, salmon, and also the main pigment in shrimp shell waste. Astaxanthin possesses antioxidant activity that able to suppress oxidative stress caused by hyperglycemia on β – pancreas cell. Shrimp shell waste can be utilized to produce astaxanthin to minimalize waste production. This study aims to find out the ability of shrimp shell crude extract activity in decreasing blood glucose level in rats induced diabetes. Shrimp shell was extracted using the maceration method with ethanol 70% as solvent. Thin-layer chromatography was used as semi – qualitative analysis method continued by spectrophotometry as a quantification analysis. Bioassay test was conducted using *Rattus norvegicus* rat conditioned with diabetes induced by Streptozotocin. Shrimp shell crude extract was given per oral every day for one month. Measurement of blood glucose level conducted using glucometer every once a week. The resulting yield extract is 0,039%. Thin-layer chromatography show 3 spot with canthaxanthin, astaxanthin monoester and β – carotene as a compound assumption. A blood glucose reduction test with a dose of 37,5 mg/kg BW show the best result. Average blood glucose level could be decreased from 429,8 mg/dL to 140,6 mg/dL.

**Keyword:** Antioxidant, Astaxanthin, Blood glucose, *Litopennaeus vannamei*, Oxidative Stress.



## INTRODUCTION

Diabetes mellitus is a not contagious chronic disease caused by the pancreas inability to produce enough insulin or the body is unable to use insulin effectively. Type II diabetes mellitus is the most common type of diabetes. Insulin resistance caused by the enhancement of free radical inside the body that antioxidant defence mechanism decrease. Type II diabetes mellitus caused by several unhealthy factors such as lack of exercise, obesity, unhealthy diet. Type II diabetes is a condition in which the pancreas can produce insulin but at a low level or a condition where the cell receptor cannot identify the insulin being produced so that sugar cannot be used by the cell resulting in a buildup of sugar in the blood.

Astaxanthin (3, 3'-dihydroxy-β, β-carotene-4, 4'-dione) is a provitamin A carotenoid pigment commonly found in marine organisms such as algae, crustacea and fish. This compound is effective to resolve free radical, reactive oxygen, and nitrogen species. Astaxanthin is the main pigment of shrimp shell waste. High level of antioxidant capability of this compound caused by a molecular structure that has a hydroxyl group and keto at each edge of the ionone ring. Astaxanthin could decrease blood glucose level, has a neuroprotective ability, has anti-inflammation and anti-oxidative ability with a minimum side effect, therefore this compound could be used to reduced diabetes complication (Ying, 2015). Astaxanthin has antioxidant activity that could suppress oxidative stress caused by hyperglycemia on β – pancreas cell. Oxidative stress suppressed by Astaxanthin will cause stability between free radical and antioxidant, therefore lowering insulin resistance (Sayahi, 2017). Reduced insulin resistance could enhance glucose tolerance, therefore hyperglycemia could be overcome (Arunkumar, 2012).

As a maritime nation, Indonesia has a lot of marine natural resources. One of the abundant marine natural resources is the crustacean group or shrimps. Shrimp is one of the most important commodities and Java Island is the biggest contributor with 28.25% production value. One of the most abundant shrimp in Indonesia is Vannamei shrimp (Liopenaeus vannamei) or the White Leg Shrimp. Vannamei shrimp used in this research are from cultivation in Gunung Kidul, Yogyakarta. The process of processing shrimp as food

generally will produce or leave some part, namely the shell, head, and tail. Unused shrimp shell will become waste from the processing. Shrimp shell possesses active compound i.e. Astaxanthin. Astaxanthin which is contained in shrimp shell could be used as basic material for alternative medicine. This study aims to study the ability of crude shrimp shell extract in lowering the blood glucose level in diabetic–induced rats.

## MATERIAL AND METHODS

### Shrimp Shell Waste Preparation

The shrimp shell separated from the flesh and cleaned up with running water then dried using the oven at 40 ºC for approximately a day. The dried sample then smashed into powder and then weighed.

### Extraction

377 g mashed and dried shrimp shell extracted using the maceration method with ethanol 70% as the solvent with 1: 10 ratio for 3 days then remacerate two times for another 3 days each. Evaporation was done using a rotary evaporator at 5 rpm with 40ºC continued with the oven at 40ºC until it forms a paste.

### Thin-layer Chromatography Analysis

The stationary phase used is Merck's TLC silica gel plate with 1 cm width and 10 cm length. Petroleum Ether and Ethanol 70% (8:2) used as the solvent. Anisaldehyde reagent was used as the spray reagent. The formed stain then observed under UV light and the Rf valued were measured.

### Quantification Analysis of Astaxanthin Content

Quantification was used using Spectrofotometry UV-Vis with wavelength 477 nm. The standard curve was created using 5 concentrations of 0.6 ppm, 1 ppm, 1.4 ppm, 1.8 ppm, and 2.2 ppm by diluting Sigma – Aldrich standard Astaxanthin from Haeatococcus pluvialis. The extract sample to be measured was made into a stock solution with a concentration of 5 ppm, and then from the stock solution, a 2.2 ppm solution was made to measure the absorbance. The resulting absorbance then calculated using the regression equation from the standard curve.

### Preclinical Test

This experimental protocol was approved under ethical clearance 1265/C.16/FK/2021 by Komisi Etik Penelitian Kesehatan FK UKDW. The animal used in this research are 24 male white Rattus norvegicus aged 8 – 12 weeks with a bodyweight of 180 – 200 g. Animals were acclimatized for seven days, with free access to water and food. Standard pellet and water were given ad libitum. After seven days, animals divided into four groups, six animals in each group. Group one as control one consist of animal with the normal condition and receive only aqua dest. Group two as control two consists of animal with diabetes-induced and receive only aqua dest. Group three as treatment one consists of animal with diabetes-induced and receive shrimp shell extract with 25 mg/kgBW dose. Group four as treatment two consist of animal with diabetes-induced and receive shrimp shell extract with 37.5 mg/kgBW. Aqua dest and shrimp shell extract were administer peroral (p.o) using gavage every day for one month.

### Diabetes Induction

Cayman chemical Streptozotocin with 50 mg/kgBW dose diluted in Citrate Buffer with 4.4 pH ; 0,1M intraperitoneally (i.p). Blood glucose level was measured after 72 hours. Animals with blood glucose level ≥ 150 mg/dL used as test animal.

### Blood Glucose Level Measurement

Blood glucose level was measured using One Touch Ultra plus Glucometer. Blood glucose level was measured once a week for one month. Blood sample was collected from vena caudalis.

## DATA ANALYSIS

Blood glucose measurement data were tabulated and analyze using One – Way Anova test by IBM SPSS Statistic 25.

## RESULT

### Sample Preparation and Extraction

Dried and mashed shrimp shell has 377 g of weight. The total weight of crude extract weight is 30 g. A crude extract from the first maceration is the weighest with 15 g and the lowest weight resulted in the third maceration with 4 g. The resulting yield from total maceration is 0.07%.

**Table-1: The Weight of Crude Extract from 377 g Dried and Mashed Shrimp Shell**

| Maceration | Crude Extract Weight (g) |
|---|---|
| Maceration I | 15 |
| Remaceration I | 11 |
| Remaceration II | 4 |
| Total | 30 |

**Table-2: Resulting Yield from**

| Dried and Mashed Shrimp Shell (g) | Crude Extract Weight (g) | % Yield |
|---|---|---|
| 377 | 30 | 0,07 % |

**Thin-Layer Chromatography**

The result of Thin Layer Chromatography analysis shows three spots with Rf value for each spot are 0.43; 0.56 and 0.93. Based on a comparison to Lorenz Todd (1998) Rf table, these spots are presumably compound from the Terpene group, that is Canthaxanthin, Astaxanthin monoester and β – carotene.

**Astaxanthin Measurement**

The highest Astaxanthin content from the measurement is 0.52 mg on maceration II crude extract. The lowest resulting content is from the first maceration with 0.36 mg. The average total astaxanthin content is 1, 32 mg. Remaceration II result is being used on the pre-clinical test.

**Table-3: Astaxanthin Content Result**

| Sample | Absorbance (Å) | Total Astaxanthin Content | Average Astaxanthin Content (mg) |
|---|---|---|---|
| Maceration I | 0,005<br>0,005<br>0,005 | 0,36<br>0,36<br>0,36 | 0,36 ± 6,79 |
| Remaceration I | 0,008<br>0,008<br>0,007 | 0,44<br>0,44<br>0,44 | 0,44 ± 0 |
| Remaceration II | 0,009<br>0,009<br>0,009 | 0,52<br>0,52<br>0,52 | 0,52 ± 0 |
| Mean | | | 1,32 ± 0,08 |

**Preliminary blood glucose level measurement**

Preliminary blood glucose measurement shows the average blood glucose level is 88.04 mg/dL which are normal and still within the range that is 50 – 135 mg/dL. Twenty-one rats (87.5%) has the normal blood glucose level, while three rats (12.5%) has abnormal blood glucose level with two rats has blood glucose level below 50 mg/dL and one rat with blood glucose level above 135 mg/dL.

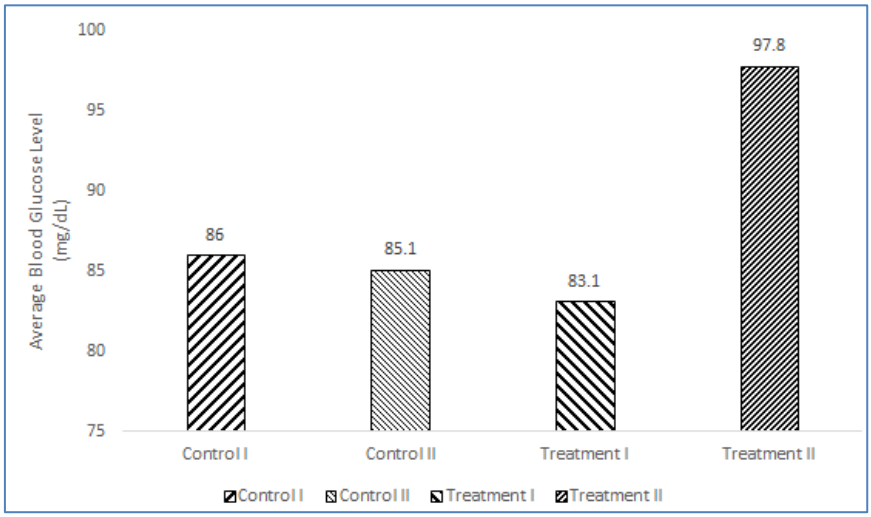


**Fig-1: Preliminary Blood Glucose Level Measurement**

Keterangan: Kontrol I (Hewan uji kondisi normal tanpa perlakuan terapi), Kontrol II (Hewan uji dalam kondisi diabetes tanpa perlakuan terapi), Perlakuan I (Hewan uji dalam kondisi diabetes dengan terapi ekstrak kasar kulit udang 25 mg/kgBB), Perlakuan II (Hewan uji dalam kondisi diabetes dengan terapi ekstrak kasar kulit udang 37,5 mg/kgBB),

**Diabetic Model on Test Animals**

Blood glucose level after 72 hours of Streptozotocin administration shows increasing in blood glucose level. On control II group show 528 mg/dL average blood glucose level with one rat died. The treatment I group show 369.7 mg/dL average blood glucose level with two rats died. Treatment II group show 429.8 mg/dL average blood glucose with one rat died. Based on the blood glucose average for each group, with blood glucose level above 150 mg/dL, it can be concluded that the diabetic model successfully created.

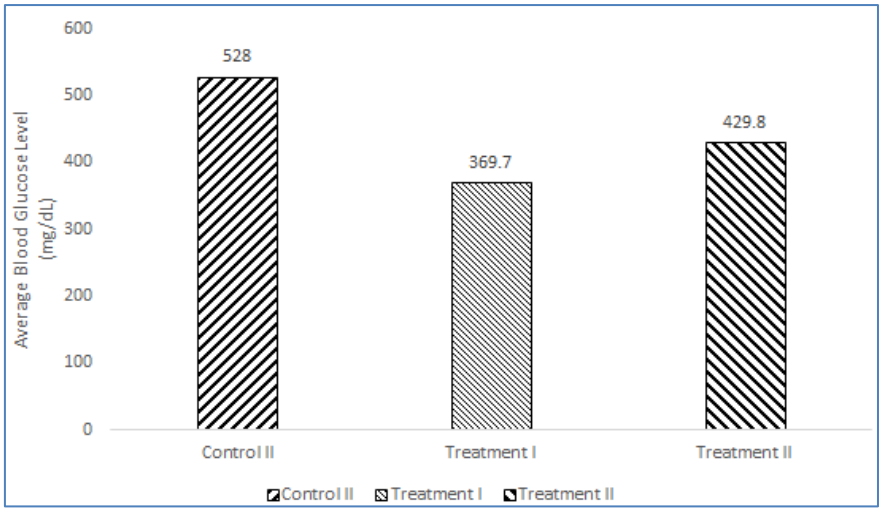


**Fig-2: Blood glucose measurement after Streptozotocin administration**

**Blood glucose level measurement once a week for one-month treatment**

Blood glucose level measurement after one-week treatment shows a decreasing blood glucose level in the control II group, treatment I group, and treatment II group. On week two, blood glucose level on control II group increase significantly ($P<0.05$) from 172 mg/dL to 540 mg/dL, while treatment I and treatment II group doesn't increase or decrease significantly ($P>0.05$).

Blood glucose level measurement after week three shows no different result from week two. Control I group shows every rat on normal condition. Control II group shows average blood glucose still at the heavy diabetic condition at 429 mg/dL while treatment I group show increasing blood glucose level from 218 mg/dL to 238 mg/dL, and treatment II group shows decreasing blood glucose level from 169 mg/dL to 159 mg/dL.

Blood glucose level measurement after week four show no different result from week two and three. The blood glucose level in the control I group are still in normal condition. The blood glucose level in the control II group shows an increasing level from 429 mg/dL to 532 mg/dL. Treatment I group show decreasing level from 238 mg/dL to 227 mg/dL and Treatment II group show decreasing level from 159 mg/dL to 140 mg/dL.

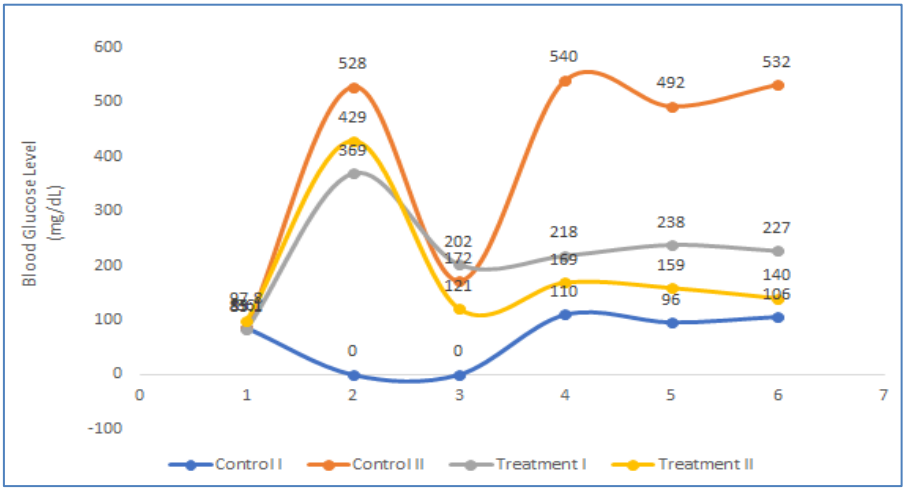


**Fig-3: One Month Measurement of Blood Glucose, 1 (Preliminary blood glucose level), 2 (Blood glucose level after induction of Streptozotocin), 3 (1st Week), 4 (2nd Week), 5 (3rd Week), 6 (4th Week)**

**Average blood glucose level after one-month treatment**

The average blood glucose level after one-month show blood glucose in the control I group are 106.83 mg/dL which categorized as normal. Control II group show blood glucose level 532 mg/dL that categorized as a heavy diabetic condition. The treatment I group show a blood glucose level of 227.2 mg/dL that categorized as a medium diabetic condition. Treatment II group show blood glucose level 140.6 mg/dL that almost reach normal condition.

ANOVA test result shows a significant difference between each group ($P<0.05$). Post hoc test Bonferroni shows a significant difference between the control II group and control I group and all treatment

group. Control I group doesn't have a significant difference against treatment I and II group. The treatment I group doesn't have a significant difference from the treatment II group.

**Table-4: Bonferroni Test Statistics Result**

| Treatment Group | | Mean Difference | Sig. |
|---|---|---|---|
| CI | CII | -425,367* | 0,000 |
| | TI | -120,417 | 0,484 |
| | TII | -33,767 | 1,000 |
| CII | CI | 425,367* | 0,000 |
| | TI | 304,950* | 0,002 |
| | TII | 391,600* | 0,000 |
| TI | CI | 120,417 | 0,484 |
| | CII | -309,950* | 0,002 |
| | TII | 86,650 | 1,000 |
| TII | CI | 33,767 | 1,000 |
| | CII | -391,600* | 0,000 |
| | TI | -86,650 | 1,000 |

*significant mean difference at 0.05

Control group II, treatment I group and treatment II group has an increased blood glucose level after streptozotocin induction. After one month of crude extract treatment, the treatment I and II group blood glucose level has a significantly lower level that is treatment I blood glucose decreased from 369 mg/dL to 227 mg/dL and treatment II group blood glucose decreased from 429 mg/dL to 140 mg/dL.

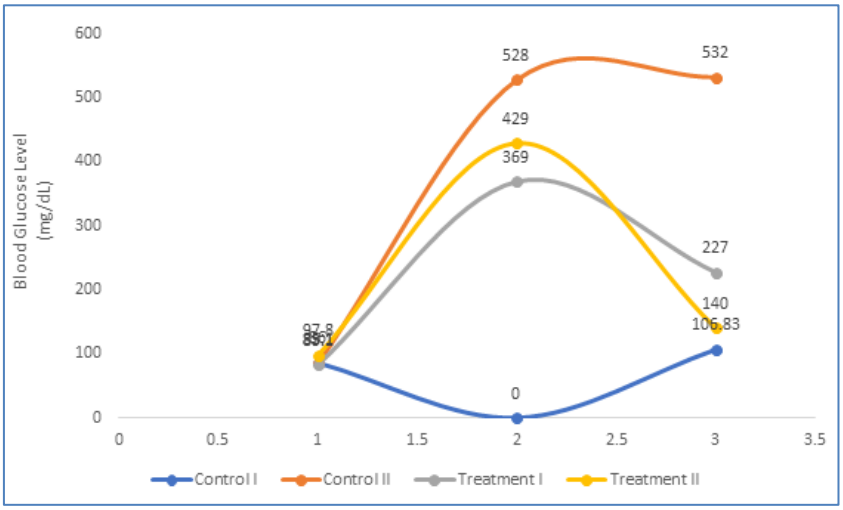


**Fig-4: Blood glucose level after Streptozotocin induction and after crude extract administration, 1 (Preliminary blood glucose level), 2 (Blood glucose level after induction of streptozotocin), 3 (Blood glucose level at the end of the research)**

## DISSCUSION

### Shrimp Shell Extraction

Maceration used as cold extraction with goals to minimize the chance of compound degradation by heat. Astaxanthin could be degradated at temperature above 60°C, therefore cold maceration such as maceration is chosen (Putri, 2019). Graded maceration method intends to maximalized the extraction process and expect a lot of compound extracted. Remaceration, as part of graded maceration conducted every two times every three days, therefore it is needed nine days to complete the whole extraction process.

Crude extract weight degradation can be seen for each remaceration. Degradation of crude extract weight can be caused by amount of crude extract have already extracted before, therefore the next remaceration process will reduce the amount of crude extract content, since it is already extracted before (Table 1).

The resulting yield is 0.07%. Faradilla (2020) extracted Acetes shrimp using the maceration method with aceton as its solvent, and the resulting yield are 11%. Ethanol 70% selected based on its semipolar characteristic and harmless. Ethanol solvent is organic solvent that could extract astaxanthin besides aceton, hexan, and methanol (Dalei, 2015).

### Thin-layer Chromatograhpy

Thin-layer chromatography analysis intends to identify presumption of compound inside the crude extract. Astaxanthin monoester and β – carotene belongs to the Terpene group. Astaxanthin monoester is a

compound derivate from Astaxanthin that could be found in shrimp (Asker, 2018). Astaxanthin monoester content on shrimp could reach 40%. β – carotene is one of the carotenoid group inside the shrimp. Therefore, it can be concluded that the presumed obtained compound is the compound be found in shrimp.

**Measurement of Astaxanthin content using spectophotometry**

The result of astaxanthin measurement shows increasing concentration for each remaceration process and the second remaceration shows the highest astaxanthin total of 0.52 mg. Increasing concentration could be caused by a lot of astaxanthin amount on the last remaceration. The weightest crude extract on the first maceration has the less amount of astaxanthin concentration. This could be caused by the first maceration did not extract the astaxanthin, but other compound i.e. fat or other compound. On the contrary, astaxanthin extracted at the last remaceration, therefore the concentration of astaxanthin is higher in the last remaceration.

**Preliminary Blood Glucose Measurement**

Preliminary blood glucose level measurement intends to find out the normal blood glucose level before given the treatment. Normal blood glucose level on rats ranged on 50 – 135 mg/dL. Based on the average blood glucose level result, it shows all rats are on normal level.

**Pre – clinical test**

Crude extract treatment with 25 mg/kgBW dose and 37.5 mg/kgBW dose is given to each treatment I and II group after the diabetic model formed. The treatment given every day for one month and blood glucose measured every once a week for one month. Decreasing blood glucose level on the first week after the diabetic model confirmed, could be caused by impermanent hyperglycemia effect that caused by difference receptor work form streptozotocin in each individual. Glucose transport to cell, forming of glucose by cell, glucose absorption of digestive tract determined the glucose concentration inside the blood (Auroma et al., 2006).

Control II group, treatment I group and treatment II group has a high blood glucose level or hyperglycemia condition after streptozotocin induction caused by β – pancreas cell damaged by reactive free radical and decreasing antioxidant defense mechanism. Average blood glucose result shows treatment II group has the lowest blood glucose level compared than treatment I group. This could be caused by higher dose on treatment II group compared by treatment I group. Different from blood glucose level on treatment I and II group, control II group show no difference on decreasing blood glucose level. Based on blood glucose level difference between control II group and treatment I and II group, it can be presumed that there is an effect of the crude extract toward the decreasing of blood glucose level.

Wang (2011) shows decreasing blood glucose result from rats induced alloxan with astaxanthin extract treatment with dose of 5 mg/kgBB and 10 mg/kgBB. Uchiyama (2002) shows the same result, with hyperlycemia condition on non – treatment group, meanwhile the treatment group has a lowered blood glucose level compared to the non – treatment group. Astaxanthin has antioxidant that able to lowered the oxidative stress caused by hyperglycemia on β – pancreas cell (Uchiyama, 2002). Astaxanthin could alleviate insulin sensitivity thus reducing insulin resistance and increasing glucose tolerance therefore hyperglycemia could be reduced.

Based on reduced blood glucose result, and other research conducted, shrimp shell crude extract has a potential ability to reduced hyperglycemia indirectly. Astaxanthin has 10 times antioxidant ability compared to other carotenoids (Sayahi, 2017). Becaused the high level of antioxidant, astaxanthin could reduce oxidative stress caused by hyperglycemia therefore insulin resistance could be reduced. This result strengthened by IPGGT test that blood glucose level on hyperglycemia treatment rats is lower than hyperlycemia non – treatment rats within two hours of treatment. This result shows increasing glucose tolerance on treatment rats indicated working insulin. Furthermore astaxanthin treatment increased stimulation on β – tyrosine insulin phosphorylase receptor and IRS-1 that connect with PI3-Kinase, pAkt/Akt and GLUT-4 translocation on skeletal muscle. It can be concluded that astaxanthin could relieve disfunction insulin response and acr as insulin activator. Therefore, on the outline, the mechanism of reducing blood glucose level with shrimp shell crude extract started by lowered oxidative stress caused by antioxidant ability. Lowered stress oxidative caused the insuline to be sensitive and lowered the insuline resistance. Successfully decreasing insulin resistance will cause the glucose tolerant to be alleviate therefore hyperglycemia could be lowered.

## CONCLUSION

Shrimp shell crude extract from shrimp shell waste could be utilized as astaxanthin sources. Dose of 37.5 mg/kgBW are the best doses and could lowered the high blood glucose level on rats – induced diabetes by streptozotocin.

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