



## The Influence of Giving Biscuits of Yellow Pumpkin Seed and Capsule of Moringa Leaves on the Level of C-Reactive Protein on Pregnant Women

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<https://doi.org/10.18280/ijdne.170419>

### ABSTRACT

**Received:** 12 July 2022

**Accepted:** 19 August 2022

#### Keywords:

*C-Reactive Protein, biscuits of yellow pumpkin seed, capsule of moringa leaves, pregnant women*

Pregnant women are likely to have the option of receiving supplementary nourishment in the form of pumpkin seed biscuits and Moringa leaves. The purpose of this study is to see how Pumpkin Seed Biscuits and Moringa Leaf Capsules affect C-Reactive Protein (C-RP) levels in pregnant women at the Stunting Locus, Bone Regency. A Quasi-Experimental method, also known as a field trial. The Non-Randomized Pre-Test - Post Test Group Design was adopted for the research design. Participants in this study were separated into two groups: group I was given pumpkin seed biscuits and a blood-added tablet, and group II was given Moringa leaf extract capsule supplements and a blood-added tablet, with a total of 30 persons in each group. The intervention lasted 90 days and included both a pre-test and a post-test. The levels of C-Reactive Protein in group I (Pumpkin Seed Biscuits) decreased by  $-0.11 \pm 0.04$  g/mL. The decrease in C-Reactive Protein levels in group II (Moringa Leaf Extract) was  $-0.09 \pm 0.04$  g/mL. Moringa capsules ( $p=0.001$ ) and pumpkin seed biscuits ( $p=0.001$ ) had an impact on reducing C-RP levels in pregnant women. Supplemental feeding of pumpkin seed biscuits and Moringa leaf extract capsules can enhance and improve the health condition of pregnant women by lowering C-Reactive Protein levels.

## 1. INTRODUCTION

To limit the likelihood of getting illnesses during pregnancy, pregnant women and children's health must be maintained by adopting a healthy lifestyle [1]. During pregnancy, a woman's nutritional intake is doubled to meet the needs of two people (mother and fetus). These nutritional intakes include calories, protein, minerals, calcium, water, omega-3, vitamins, folic acid, and iron [2], and pregnant women's nutritional consumption has a significant impact on fetal growth [3]. Iron, also known as Ferrum in Latin and Iron in English, plays a key part in many metabolic processes as well as serving as a structural center in the production of hemoglobin (Hb) for the process of carrying oxygen [4]. Low hemoglobin levels will occur from iron shortages during pregnancy [5]. Several variables regulate iron metabolism, one of which is the protein found in the C-RP biomarker [6]. According to Namaste, 2014, numerous biomarkers of iron metabolism components, such as hemoglobin (Hb), ferritin, and C-Reactive Protein, can be used to measure iron status (C-RP) [7].

Elevated CRP levels during pregnancy have been linked to complications such as preeclampsia and intrauterine growth restriction. According to Musrifah's research, pregnant women with preeclampsia have higher C-RP levels than pregnant women without preeclampsia. The mean CRP in pregnant

women with preeclampsia ( $4.11 \pm 0.37$  mg/dl) was higher than in normal pregnant women ( $2.49 \pm 0.26$  mg/dl) and higher than in women who were not pregnant ( $1.33 \pm 0.15$  mg/dl) [8]. C-RP activates neutrophils and inhibits platelet aggregation by binding directly to bacteria as an opsonin for a complement. Elevated C-RP levels during pregnancy are associated with an increased risk of fetal growth restriction and neonatal complications, such as premature birth and low birth weight [9]. C-RP activates neutrophils and inhibits platelet aggregation by binding directly to bacteria as an opsonin for a complement. Increased C-RP levels during pregnancy are linked to an increased risk of fetal growth restriction and newborn problems such as preterm delivery and low birth weight [10]. Research in Indonesia discovered that, in addition to anemia caused by iron deficiency in pregnant women, low birth weight is the most significant risk factor for stunting [11].

Basic Health Research shows that the national prevalence of stunting is 37.2% which indicates an increase in cases compared to 2010 with a case percentage of 35.6% and in 2007 the percentage of cases was 36.8% [12]. The prevalence of stunted toddlers has increased from 2016, from 27.5% to 29.6% in 2017. In South Sulawesi, the highest stunting was found in Bone Regency with a percentage of 40.1% (2017), and 37.3% (2018). Therefore, South Sulawesi has designated the two districts as stunting loci [13].

Pumpkin fruit is a type of fruit in the *Cucurbitaceae* family [14]. Pumpkin is widely grown in Africa, America, India, and China [15, 16]. Pumpkin is a plant that is common in Indonesia [17]. Pumpkin seeds can increase nutritional status, body weight, and zinc levels [18]. However, the usage of pumpkin seeds in the community is still quite uncommon. Pumpkin Seeds' nutritional potential can be turned into a dietary product for pregnant women. 100 grams of pumpkin seeds, on the other hand, provide 446 calories, 18.55 grams of protein, 19.4 grams of fat, 53.75 grams of carbs, 3.2 grams of Omega-3, and 23.4 grams of Omega-6 [19]. Pumpkin seeds are used in the manufacture of food items by processing pumpkin seed flour into a variety of foods such as cookies, cake, panjeeri, laddoo, and math. The nutritious value of all sorts of meals that had been supplemented with pumpkin seeds flour was higher than those that had not been combined with pumpkin seeds [20].

In addition to pumpkin seeds, Moringa leaves are known as *Folia Moringa* in Latin and *Moringa Leaves / M. Oleifera* in English. administration of Moringa leaf extract was linked with increased body weight and contributed to an increase in upper arm circumference in pregnant women [21]. Another research in Jeneponto discovered that Moringa leaf extract and flour can boost hemoglobin levels in anemic pregnant women [22]. Moringa leaves contain very high amounts of vitamin A, vitamin C, vitamin B, calcium, potassium, iron, and protein, which are easily digested and assimilated by the human body [23, 24].

Several studies have reported that the intervention of Moringa leaves has been used to overcome nutritional problems, especially in pregnant women, nursing mothers, and children, where Moringa leaves contain high protein and micronutrients [25, 26]. Countries such as Senegal, India, Benin, and Zimbabwe have also used Moringa leaves as a strategic step for nutrition improvement actions through malnutrition prevention programs [27]. In Thailand, Moringa leaves are considered an excellent daily protein supplement [28]. In addition, moringa-based supplements showed that moringa extract could reduce DNA damage in pregnant women, significantly increasing hemoglobin concentrations in anemic women [29].

This study aims to determine the effect of giving Pumpkin Seed Biscuits and Moringa Leaf Capsules to C-Reactive Protein (C-RP) levels in pregnant women at the Stunting Locus, Bone Regency. The results of this study help the government in supporting the program to improve the health of pregnant women by preventing anemia and preventing implantation in pregnant women.

## 2. METHODS

A Quasi-Experimental method, commonly known as a field experiment (field trial). The Non-Randomized Pre Test - Post Test Group Design was adopted for the investigation. In this study, participants were placed into two groups of 30 persons each: group I was given pumpkin seed biscuits + blood-added tablets, while group II was given Moringa leaf extract capsules + blood-added tablets. The intervention lasted 90 days and included both a pre-test and a post-test. Group I consists of pregnant women living in Bone Regency, where 14 villages serve as research sites. For three months, pregnant women in their first and second trimesters were given four pieces of pumpkin seed biscuit (2 pieces in the morning and two pieces in the afternoon). Group II consists of pregnant women in their

first and second trimesters who live in as many as 14 villages in the Bone area and are given Moringa pills once a day. C-Reactive Protein (C-RP) levels in blood were measured before and after the intervention.

### 2.1 Implementation of intervention

The intervention lasted three months or 90 days, with the research sample randomly divided into two groups: group A (Pumpkin Seed Biscuits) and group B (Pumpkin Seed Biscuits) (Moringa leaf extract capsules). Field officers delivered biscuits and Moringa capsules to both groups once a week, at a dose of 4 pieces per day for group A and one capsule of Moringa leaf extract per day for group B. Field officers observed the intervention by inquiring and verifying the number of remaining biscuits and Moringa extract capsules and reminding participants to eat the biscuits and take the capsules regularly. Pumpkin seeds (*Cucurbita Sp.*) are a raw material used in the production of biscuits derived from pumpkin grown in Makassar and its surrounds. Pumpkin seeds were dried in the sun for around 7 hours before being roasted at a temperature of 80-100°C for 2 hours. After roasting the oven seeds, they were mixed till smooth and sieved through a 32 mesh sieve to achieve a flour form. This flour forms the base for these biscuits, which also include wheat flour, egg yolks, refined sugar, margarine, baking soda, and vanilla extract [30].

Moringa leaf material utilized in this study was the shoots of Moringa leaves up to the seventh stalk, which was then cleaned by immersing them in water and flushing them numerous times with running water. After washing, the Moringa leaves were aerated for around 2 hours before being threshed off the stalks. After that, they were dried on a clothesline using an incandescent bulb set to 38-39°C for two days or until they looked dry. The dried leaves were kneaded until they were little using covered hands. The half-fine Moringa leaves were transferred to the UNHAS Research Activity Center's Micronutrient Laboratory and extracted in capsules weighing 600 mg/capsule.

### 2.2 Data analysis

We used paired t-test to see the effect of Pumpkin Seed Biscuits Moringa and Leaf Extract Capsules on C-RP levels and an independent-sample t-test to see the different effects between Pumpkin Seed Biscuits Moringa and Leaf Extract Capsules. We used an alpha of 0.05 as the significance level. The data was analyzed using SPSS version 21 software.

### 2.3 Ethical aspects

Ethical clearance was approved by the Research Ethics Commission of the Faculty of Public Health, Hasanuddin University. In conducting research, informed consent is given to be signed to respondents before.

## 3. RESULTS

The results of the analysis found that there was no significant difference related to age ( $p=0.743$ ), body weight ( $p=0.111$ ), height ( $p=0.204$ ), BMI ( $p=0.260$ ), and gestational age ( $p=0.588$ ) between the control group. (Kelor Leaf Extract) and the Intervention group (Pumpkin Seed Biscuits) so that the

data can be classified as homogeneous (Table 1).

The levels of C-Reactive Protein in group I (Pumpkin Seed Biscuits) decreased by  $-0.11 \pm 0.04$  g/mL and based on the results of the Paired T-Test statistical test, it was found that the effect of giving Pumpkin Seed Biscuits to pregnant women on the decrease in C-Reactive Protein levels was found. ( $p < 0.001$ ). The decrease in C-Reactive Protein levels in group II (Moringa Leaf Extract) was  $-0.09 \pm 0.04$  g/mL where the

results of the Paired T-Test statistical test revealed that there was an effect of giving Moringa capsules to pregnant women to decrease C-Reactive Protein levels ( $p < 0.001$ ) (Table 2 and Figure 1). The results of the independent statistical test sample t-test found no difference in the levels of C-Reactive Protein ( $p = 0.292$ ) between group I (Yellow Pumpkin Seed Biscuits) and group II (Moringa Leaf Extract) (Table 3 and Figure 2).

**Table 1.** Characteristics of respondents

Characteristics	I (Pumpkin Seed Biscuits)		II (Moringa Leaf Extract Capsules)		p
	Mean±SD	Median	Mean±SD	Median	
Age (year)	25.50±5.13	24.50	25.93±5.05	24.50	0.743*
Body Weight (kg)	51.93±9.55	48.95	56.00±9.91	54.50	0.111*
Height (m)	151.07±5.40	151.50	152.97±6.03	154.00	0.204*
BMI (m/kg <sup>2</sup> )	22.76±3.97	21.88	23.91±3.87	23.02	0.260*
Gestational Age (week)	17.93±5.15	20.00	17.13±6.16	20.00	0.588**

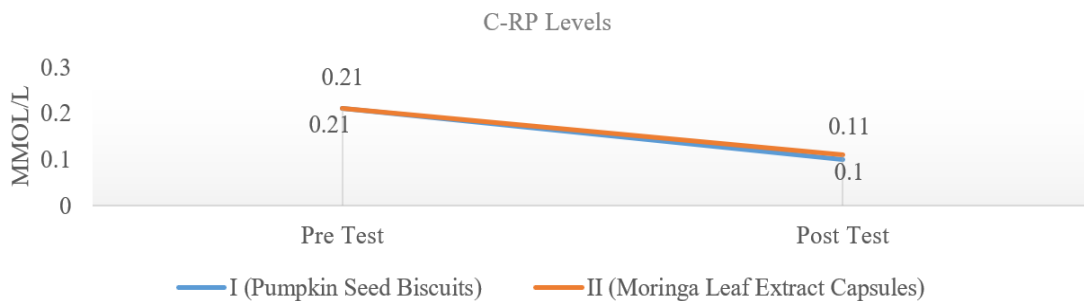
\* Independent Sample T Test, \*\*Mann Whitney

**Table 2.** The effect of giving pumpkin seed biscuits and moringa leaf extract capsules to pregnant women on C-reactive protein (C-RP) levels (n:60)

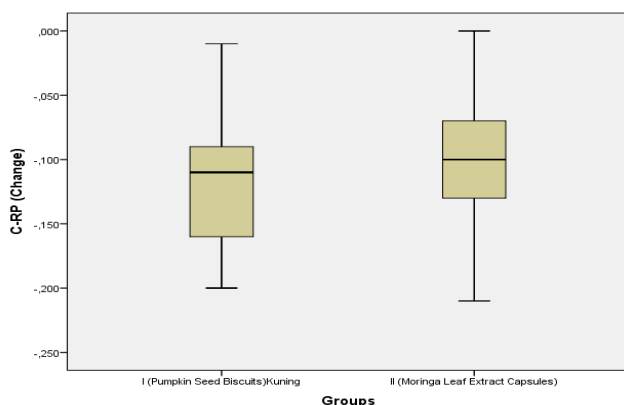
Variables	I (Pumpkin Seed Biscuits)			II (Moringa Leaf Extract Capsules)		
	Pre Test	Post Test	p Value	Pre Test	Post Test	p Value
C-RP levels (µmol/l)	0.21±0.03	0.10±0.04	<0.001	0.21±0.02	0.11±0.04	<0.001

**Table 3.** Differences in changes in C-RP levels between giving pumpkin seed biscuits and moringa leaf extract capsules to pregnant women

Variables	n	Mean±SD			
		I (Pumpkin Seed Biscuits)	II (Moringa Leaf Extract Capsules)	Different	p Value
Change of C-RP levels (µmol/l)	30	0.11±0.04	0.09±0.04	0.01±0.01	0.292



**Figure 1.** C-RP levels



**Figure 2.** Changes in C-RP levels

#### 4. DISCUSSION

The results of this study revealed a decrease in the levels of C-Reactive Protein where the levels of C-RP in the intervention group (Yellow pumpkin seed biscuit) was  $0.21 \pm 0.03$  g/mL and in the control group (Moringa Leaf Extract) was  $0.21 \pm 0.02$  g/mL. At the post-test, the lowest levels of C-Reactive Protein were found in the Control group (Moringa Leaf Extract) ( $0.10 \pm 0.04$  g/mL) compared to the Intervention group (Yellow Pumpkin Seed Biscuits) ( $0.10 \pm 0.04$  g/mL).

C-RP is a more sensitive biomarker of bacterial infection, inflammation, and tissue damage than other acute-phase proteins, although it is not specific for inflammatory tissue damage. This is since the amount of C-RP in the serum must be determined by displaying a picture of the disease and its clinical symptoms. Normal pregnancy might result in

inflammatory responses. Normal pregnancy, on the other hand, has a lesser inflammatory response than preeclampsia. The idea of syncytiotrophoblast micro fragments in the maternal circulation explains this. C-RP may be identified in normal gestational plasma, but its concentration rises dramatically in preeclampsia. C-RP is an inflammatory protein that has been linked to endothelial dysfunction [31].

The results of this study found a decrease in the levels of C-Reactive Protein in group I (Yellow Pumpkin Seed Biscuits) by  $-0.11 \pm 0.04$  g/mL. The results of the Paired T-Test statistical test also found that there was an effect of giving Pumpkin Seed Biscuits to pregnant women on decreasing levels of C-Reactive Protein ( $p < 0.001$ ). Research conducted by Maiya (2017) in Texas reported that the group given pumpkin seed oil and the group given pumpkin seeds did not experience a significant increase (anthropometry, plasma lipids, CRP and EndoPAT). Although not significant, there were a slight increase in C-reactive protein (CRP) levels [32]. Before being given pumpkin seed oil, the average CRP value in the group was  $2.49 \pm 2.20$  and after 12 weeks of administration, the average CRP value was  $2.07 \pm 1.70$ . Meanwhile, in the group that was given pumpkin seeds, the average CRP was  $1.29 \pm 1.13$  before being given pumpkin seeds and became CRP  $1.33 \pm 1.18$  after 12 weeks of administration [32]. Atherogenic mice showed a significant increase ( $p < 0.001$ ) in serum arginine levels compared to matched control mice (16.54 and 9.23 mg/dl, respectively). Control mice fed 2% arginine or pumpkin seed supplements showed a significant ( $p < 0.001$ ) reduction in serum arginine concentrations. In contrast, no significant changes were seen in serum arginine levels of atherogenic mice treated. Serum C-RP levels in atherogenic rats were not significantly different from control rats. Supplementation with 2% arginine or pumpkin seeds had no significant effect on serum C-RP of the treatment group when compared to matched controls [33].

Pumpkin seeds contain oil (41.59%), protein (25.4%), moisture (5.2%), carbohydrates (25.19%), fiber (5.34%) and total ash (2.49%). Total phenolic compounds, total sterols, and total tocopherols were 66.25 (mg gallic acid per kg oil), 1.86%, 1.56%, and 882.65 (mg tocopherol per kg oil) [34]. Pumpkin seed biscuits for pregnant women include 192.5 kcal total calories and 107.01 kcal energy from fat per serving (36 g). Pregnant pumpkin seed biscuit PMT has 17.34 grams of carbs, 11.89 grams of fat, 4.030 grams of protein, and 0.59 grams of sodium and fiber per serving. Vitamin A, Vitamin C, Calcium, Potassium, Zinc, Fe, and Flavonoids are all found in PMT Pregnant Pumpkin Seed Biscuits. Provisions for distributing Pumpkin Seed Biscuits to PMT Pregnant women are given four pieces each day, two in the morning and two in the afternoon.

The results of this study showed that the levels of C-Reactive Protein in group II (Moringa Leaf Extract) decreased by  $-0.09 \pm 0.04$  g/mL. According to the findings of the Paired T-Test statistical test, providing Moringa capsules to pregnant women affected reducing C-Reactive Protein levels. ( $p < 0.001$ ). The leaves of *M. oleifera* are rich in minerals such as calcium, potassium, zinc, magnesium, iron, and copper. Vitamins like beta-carotene vitamin A, B vitamins like folic acid, pyridoxine, and nicotinic acid, and vitamins C, D, and E are also present in *M. oleifera*. Phytochemicals such as tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones, alkaloids, and reducing sugars are present alongside anti-cancer agents such as glucosinolates, isothiocyanates, glycosides compounds and glycerol-1-9-octadecanoic. Moringa leaves

also have a low calorific value and can be used in the diet of those who are obese [35]. Antioxidants are substances that help protect the body's cells from free radical damage. Moringa includes 46 strong antioxidant molecules that protect the body from free radical cell damage. Moringa has 46 potent antioxidants. The antioxidant compounds contained in Moringa are Vitamin A with an amount of 10 times more than Beta Carotene with a large content of 7013 IU, Vitamin C as much as 220 mg/100 g fresh leaves, Vitamin E as much as 113 mg/100 g leaf powder, Vitamin K as much as 108 g/100 g of dried leaves, Vitamin B (Choline) as much as 423 mg. 100 g of fresh leaves, Vitamin B1 (Thiamin) as much as 2.6 mg/100 g of dry leaves, Vitamin B2 (Riboflavin) as much as 20.5 mg/100 g dried leaves, Vitamin B3 (Niacin) as much as 8.2 mg/100 g dry leaves, Vitamin B6 as much as 1200 mg. 100 g fresh leaves, Alanine as much as 15262.89 Ppm, Arginine as much as 406.6 mg/100 g fresh leaves, Bet -Sitosterol, Caffeoylquinic Acid, Campesterol, Carotenoids, Chlorophyll, Chromium, Delta-5-Avenasterol, Delta-7-Avenasterol, Glutathione, Histidine, Indole Acetic Acid, Indoleacetonitrile, flavonoids, Kaempferol, Leucine 492.2 mg/10 gr leaves fresh, Lutein, Methionine as much as 117.7 mg/100 gr fresh leaves, Myristic-Acid, Palmitic-Acid, Prolamine, Pr oline as much as 19607.69 ppm, Quercetin, Rutin, Selenium as much as 0.10 mg/kg, Threonine as much as 117.7 mg/100 g fresh leaves, Tryptophan as much as 107 mg/100 g fresh leaves, Xanthins, Xanthophyll, Zeatin, Zeaxanthin, Zinc as much as  $< 0.006$  mg/kg [36].

According to the findings of the independent statistical test sample t-test, there was no difference in changes in C-Reactive Protein levels ( $p: 0.292$ ) between groups I (Yellow Pumpkin Seed Biscuits) and II (Kelor Leaf Extract). The findings of the independent statistical test sample t-test also revealed no difference in C-RP level variations ( $p: 0.862$ ) between the Control group (Moringa Leaf Extract) and the Intervention group (Yellow Pumpkin Seed Biscuits). This demonstrates that Moringa Leaf Extract and Pumpkin Seed Biscuits have nearly the same potential for lowering C-RP levels in pregnant women, so the Gammarana program is a South Sulawesi Province community movement to prevent stunting by providing products made from natural ingredients in the form of seed biscuits. Pumpkin and Moringa pills can be utilized to improve mother and child health in the future.

The C-Reactive Protein (C-RP) test can detect infections and inflammatory issues in the laboratory. C-RP levels rise a few hours after inflammation and reach a peak in 2-3 days. The greater the stimulation, the higher and longer the C-RP levels. When the inflammatory stimulation is removed, the C-RP level rapidly declines. Elevated CRP levels during pregnancy have been linked to complications such as preeclampsia and intrauterine growth restriction [37]. According to Musrifah's research, pregnant women with preeclampsia have higher C-RP levels than pregnant women without preeclampsia. Mean CRP in preeclampsia was  $4.11 \pm 0.37$  mg/dl, while in normal pregnant women was  $2.49 \pm 0.26$  mg/dl and in non-pregnant women was  $1.33 \pm 0.15$  mg/dl [9]. The results of a systematic review found that giving additional food to pregnant women by giving pumpkin seeds and Moringa leaf extract had an impact on improving nutritional status, Hb, ferritin, and CRP levels in pregnant women, as well as preventing adverse pregnancy outcomes such as low birth weight [38].

Researchers did not utilize a negative control in this study to compare the effect of administering pumpkin seed biscuits on reducing levels of C-Reactive Protein in pregnant women.

Furthermore, because researchers did not mix pumpkin seed biscuits and Moringa capsules in this study, researchers were unable to analyze the effect of administering pumpkin seed biscuits paired with Moringa capsules on hemoglobin, ferritin, and C-Reactive Protein levels in pregnant women. Food consumption is greatly affected by household disposable income and the family structure, so a seed program is needed to utilize the yard [39]. The development and production of pumpkin and Moringa seeds through a seed program through the use of home gardens can meet the availability of pumpkin and Moringa seeds. Plant the Seed had a positive impact on factors associated with healthy eating behavior. This program demonstrates how to effectively engage community resources, to promote the development and implementation of gardening environments that create hands-on opportunities for children to become directly connected to local food production, to learn about the environmental benefits of eating locally grown foods and to allow children to have access to a positive, sustainable food environment [40]. In addition, poverty alleviation and food security will depend on government's full involvement in agriculture and improvement on its agricultural budget [41].

## 5. CONCLUSIONS

Supplemental feeding of pumpkin seed biscuits and Moringa leaf extract capsules can enhance pregnant women's health by lowering C-Reactive Protein levels. The combination of Giving Biscuits of Yellow Pumpkin Seed and Capsule of Moringa can be developed into one of the complementary foods for pregnant women to meet the nutritional needs of pregnant women throughout the reproductive life cycle of the mother and the growth and development of infants and children.

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