



The Effect of Pumpkin Seeds Biscuits and Moringa Extract Supplementation on Hemoglobin, Ferritin, C-reactive protein, and Birth Outcome for Pregnant Women: A Systematic Review

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Abstract

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BACKGROUND: According to the WHO data, 40% of maternal mortality in developing countries is related to anemia in pregnancy. Most anemia in pregnancy is caused by iron deficiency and acute bleeding, sometimes even the two interact. The incidence of anemia in pregnancy in Indonesia is quite high, which is around 67% of all pregnant women, with variations depending on each region. About 10–15% of pregnant women are classified as severe anemia which of course will affect the growth and development of the fetus in the womb. Most of the anemia in pregnant women occurs due to malnutrition.

AIM: The objective of the study was to review journals related to the effect of pumpkin seed biscuits and *Moringa* capsules on hemoglobin (Hb), ferritin, and C-reactive protein (CRP) levels in pregnant women and also the prevention of stunting.

METHODS: Study of literature by collecting relevant literature using online journal data based on PUBMED, Google Search, ELSEVIER, MDPI, DOAJ (Direct Directory of Open Access Journals), or from the bibliography of the articles being searched.

RESULTS: Supplementary food was given to pregnant women by giving pumpkin seeds and *Moringa* leaf extract 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.

CONCLUSION: The provision of pumpkin seed biscuits and *Moringa* leaf extract to pregnant women can affect Hb, ferritin, and CRP levels and can affect maternal nutritional status and affect pregnancy outcomes.

Introduction

Pregnancy is the growth and development of an intrauterine fetus from conception and ending until the onset of labor. The duration of gestation is estimated at 280 days (40 weeks) and not more than 300 days (43 weeks). According to the WHO data, 40% of maternal deaths in developing countries are related to anemia during pregnancy. Most anemia in pregnancy is caused by iron deficiency and acute bleeding, sometimes even the two interact. The incidence of anemia in pregnancy in Indonesia is quite high, which is around 67% of all pregnant women, with variations depending on each region. Iron metabolism is influenced by various factors, one of which is protein [1]. Protein has an important role in providing organic iron intake. Organic iron is obtained from food intake, especially in red meat [2], and is an important functional unit for binding iron and preventing potential toxic oxidants in the form of hemoproteins as heme compounds (hemoglobin [Hb] or myoglobin) and heme enzymes. Ferritin is an iron storage protein and is present

extracellularly in serum. Ferritin serves as a clinical marker of the status of the body's iron stores [3]. Iron deficiency anemia caused significant increase in platelet count (PT) and total iron-binding capacity (TIBC) [4].

The WHO data report that babies with low birth weight (LBW) account for 60–80% of all neonatal deaths and have a risk of death 20 times greater than babies with normal weight. The percentage of LBW in developing countries is 16.5%, twice as large as in developed countries (7%) [5]. Long-term impact of LBW is stunting. Stunting is a physical growth disorder characterized by a decrease in growth rate and is a result of nutritional imbalances. Stunting is still a nutritional problem in Indonesia that has not been resolved. Stunting will cause long-term impacts, namely, disruption of physical, mental, intellectual, and cognitive development. Children who are stunted until the age of 5 years will be difficult to repair so that it will continue into adulthood and can increase the risk of offspring with LBW [6].

Pregnant women should meet the requirement for certain nutrients, especially micronutrients to ensure

the better pregnancy outcome later on. Efforts to fulfill the nutritional status of pregnant women and babies can be fulfilled with a good source of nutrition, namely, pumpkin fruit and *Moringa* leaf. The pumpkin plant is a type of fruit that belongs to the Cucurbitaceae family, including seasonal plants that bear fruit immediately after death. Pumpkin seeds are a plant that is easy in the nursery, care, and the results are sufficient to provide high economic value to the community. Pumpkin seeds are widely cultivated in African countries, America, India, and China. Pumpkin seeds usually grow in low and high plains, altitudes between 0 m and 1500 m above sea level [7].

Pumpkin seeds are a plant that is easily found in Indonesia. However, the use of pumpkin seeds in the community is still minimal. *Cucurbita moschata* seeds contain several substances, including a type of amino acid such as m-carboxyphenylalanine, amino butyrate, and citrulline as well as a number of other amino acids needed by the prostate gland such as seminal alanine, glycine, and glutamic acid. These seeds also contain mineral elements Zn (zinc) and Mg (magnesium) which are very important for reproductive health. Pumpkin seeds are a good source of phytoestrogens and may exert week estrogenic or anti-estrogenic effects which decrease blood pressure and reduce C-reactive protein (CRP) [8]. CRP is expressed in human atherosclerotic plaques and both vascular cells and monocytes/macrophages appear to represent a significant source of CRP in the inflammatory vessel wall [9]. *Moringa oleifera* leaves have long been used to overcome the problem of malnutrition among children, pregnant women, and breastfeeding. In addition, with micronutrients substances, *M. oleifera* can be used an alternative supplement for pregnant women to prevent maternal anemia and LBW.

The aim of this literature review is to assess journal article related to impact supplementation of pumpkin seeds and *Moringa* extract on Hb, ferritin, CRP, and birth outcome for pregnant woman.

Methods

Study selection: The systematic review uses database from the PubMed, Scinapse, and ProQuest. The search criteria in the database use the word “pumpkin seeds,” “*Moringa* leaf,” “outcome pregnant,” growth with restrictions on articles published during the past 10 years (2010–2020), English language articles, open access. The inclusion criteria were as follows: (1) Full-text articles were selected, (2) targeting studies of mother- children (under 5 years old), and (3) randomized controlled trial study. Exclusion criteria were as follows: (1) Review/editorial, (2) conference proceedings, (3) systematic review/literature review, (4) study protocol,

and (5) meta-analysis. Figure 1 shows a flowchart for study based on PRISMA 2015 guidelines.

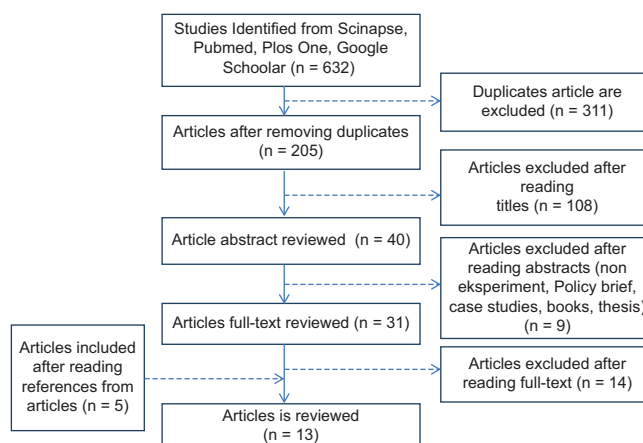


Figure 1: Flowchart for study based on PRISMA 2015 guideline

Results and Discussion

Table 1 shows that there are 13 articles that were successfully selected related to the Effect of intervention pumpkin seeds and Moringa leaf on pregnancy outcomes published in 2008 - 2019.

Pumpkin seeds and Moringa leaf extract

The chemical composition and several bioactive components such as tocopherols, carotenoids, and β -sitosterol were analyzed in three main pumpkin species (*Cucurbitaceae pepo*, *C. moschata*, and *Cucurbita maxima*) in three parts (peeled, meat, and seeds) each of the pumpkin species. *C. maxima* contains more carbohydrates, protein, fat, and fiber than *Cucurbita pepo* or *C. moschata* ($p < 0.05$). The water content and amino acids and arginine content in all parts of the pumpkin are the highest in *C. pepo*. The main fatty acids in the seeds are palmitic, stearic, oleic, and linoleic acids. The seeds of *C. pepo* and *C. moschata* contain much more γ -tocopherol than *C. maxima*, whose seeds contain highest β -carotene. *C. pepo* seeds contain much more β -sitosterol than other seeds. Nutrient composition varies different between species and parts of the pumpkin [10]. Nutritional value of pumpkin seeds per 100 g included carbohydrates 10.71 g, energy 559 kkal, total fat 49.05 gr, protein 30.23 g, fiber 6 g, cholesterol 0 mg, folic acid 58 μ g, niacin 4.8 mg, Vitamin B5 0.75 mg, B6 0.14 mg, B2 0.15 mg, thiamin 0.272 mg, Vitamin C 0.272 mg, Vitamin A 16 IU, Vitamin E 35.1 mg, mineral sodium 7.0 mg, potassium 809.0 mg, minerals cobalt 1.43 mg, iron 8.8 mg, magnesium 592 mg, mangan 4.54 mg, phosphor 1232 mg, selenium 9.40 μ g, and zinc 7.8 mg. Phytochemical dense including beta-carotenoid 9 μ g, beta-cryptoxanthin 1 μ g, and lutein zeaxanthin 74 μ g [11].

Table 1: Effect of intervention pumpkin seeds and *Moringa* leaf on outcome pregnancy

S. No.	Author/place/year	Title	Study objective	Design	Study outcome
1	Maiya M/USA/2017 [8]	Dissertation: Effect of supplementation with pumpkin seed oil versus pumpkin seeds on blood pressure and menopausal symptoms in non-hypertensive postmenopausal women	To compare influences pumpkin seeds (1 1/2 teaspoons/4.1 g daily) versus pumpkin seed oil (2 g/day) for 12 weeks on blood pressure (systolic and diastolic), endothelial function, plasma lipids, C-reactive protein concentration, and menopausal symptoms in non-hypertensive postmenopausal women	Randomized control trial	Pumpkin seeds help with weight loss and reduction in body fat percent which is most likely due to the benefits of satiety from high protein and fiber content in pumpkin seeds. Pumpkin seed oil supplementation confirmed previous findings suggest a significant reduction in diastolic blood pressure and menopause symptoms, small improvements in low-density lipoprotein and total cholesterol, and decreased C-reactive protein
2	Naghii and Mofid M/Iran/2008 [27]	Impact of daily consumption of iron fortified ready-to-eat cereal and pumpkin seed kernels (<i>Cucurbita pepo</i>) on serum iron in adult women	This is to determine the effectiveness of consuming ready-to-eat cereals and pumpkin seed kernels on status of iron nutrition and response of hematological characteristics of women at reproductive ages	Experiment study	Better response for iron status was observed after intervention. The statistical analysis showed a significant difference between the pre and post consumption phase for higher serum iron (60 ± 22 vs. 85 ± 23 ug/dl), higher transferrin saturation percent (16.8 ± 8.0 vs. $25.6 \pm 9.0\%$), and lower total iron binding capacity (367 ± 31 vs. 339 ± 31 ug/dl). A significant positive correlation ($r = 0.981$, $p = 0.000$) between the differences in serum iron levels and differences in transferrin saturation percentages and a significant negative correlation ($r = -0.916$, $p < 0.001$) between the differences in serum iron levels and differences in total iron binding capacity
3	Resmi et al. /India/2017 [16]	Effectiveness of amla, jaggery and pumpkin leaves extract on the level of hemoglobin, Vitamin C, and iron among adolescent girls with iron deficiency anemia	Assessing the effectiveness of a mixture of amla, jaggery, and pumpkin leaves on hemoglobin, Vitamin C, and iron levels in adolescent girls with iron deficiency anemia	Randomized to the control and experimental group	There was significant increment in the pre-test and post-test level of hemoglobin, Vitamin C, and iron of the subjects in the experimental group. In the experimental group, there was a rise in mean values for hemoglobin from 9.942 to 10.99, Vitamin C from 4.302 to 5.63, and iron from 77.6 to 99.58
4	Soltan SSA/Egypt/2013 [4]	The protective effect of soybean, sesame, lentils, pumpkin seeds, and molasses on iron deficiency anemia in rats	To determine the protective effect of cooked soybeans, ripe lentils, sesame seeds, pumpkin seeds, molasses, and their mixtures (soy + lentils + sesame seeds + pumpkin seed powder + molasses) with ascorbic acid against iron deficiency anemia	Randomized to the control and experimental group	Rats fed on iron-free diet supplemented with seven iron sources revealed significant increase in hemoglobin, Hct, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell, white blood cell, serum iron, and significant decreased in platelet count and total iron-binding capacity. Mixture of legumes, cereals, and molasses with ascorbic acid and molasses with ascorbic acid has protection against iron deficiency anemia and is equivalent to FeSO
5	Abuelgassim and Al-Showayman SIA/Saudi Arabia/2012 [17]	The effect of pumpkin (<i>Cucurbita pepo</i> L.) seeds and L-arginine supplementation on serum lipid	To determine the effect of pumpkin seed supplementation (<i>Cucurbita pepo</i> L.) on atherosclerosis due to an atherogenic diet	Randomized to the control and experimental group	Atherogenic rats supplemented with pumpkin seeds showed a significant decrease ($p < 0.001$) in their serum concentrations of total cholesterol and low-density lipoprotein cholesterol as they dropped from 4.89 mmol/L to 2.55 mmol/L and from 3.33 mmol/L to 0.70 mmol/L, respectively. Serum concentrations of high-density lipoprotein cholesterol were also significantly elevated in the same group. Although, atherogenic rats supplemented with 2% arginine showed significant increase in serum concentration of high-density lipoprotein-cholesterol, no significant changes were observed in their serum concentrations of total cholesterol and low-density lipoprotein-cholesterol
6	Nishimura et al., /Japan/2014 [18]	Pumpkin seed oil extracted from <i>Cucurbita maxima</i> improve urinary disorder in human overactive bladder	Evaluating effects pumpkin seed oil from <i>Cucurbita maxima</i> against dysfunction urinary tract on human overactive bladder	Experimental study	Pumpkin seed oil from <i>C. maxima</i> significantly reduced the degree of overactive bladder symptom score in the subjects. Pumpkin seed oil extracts from <i>C. maxima</i> as well as from <i>C. pepo</i> are effective for urinary disorders such as overactive bladder in humans
7	Kanwal et al., /Pakistan/2015 [19]	Development, physico chemical and sensory properties of biscuits supplemented with pumpkin seeds to combat childhood malnutrition in Pakistan	Develop high energy biscuits which is equipped with pumpkin seeds for improved nutrition and evaluate attributes chemical and organoleptic from extra biscuits	Experimental study	Biscuits fortified with nutritious pumpkin seed flour were made with four different levels of pumpkin seed flour substitution in wheat flour (T2 = 5%, T3 = 10%, T4 = 15% and T5 = 20%), compared to control (T1). The chemical attributes of biscuits show that T5 has the highest levels in pumpkin flour (20%) with the maximum protein (12.30%), fat (28.29%), ash (4.13%), iron (2.28%), and zinc (3.11%). The sensory results also show an increasing trend in all sensory parameters

(Contd...)

Table 1: (Continued)

S. No.	Author/place/year	Title	Study objective	Design	Study outcome
8	Iskandar et al., /Indonesia/2015 [20]	Effect of <i>Moringa oleifera</i> leaf extracts supplementation in preventing maternal anemia and low birth weight	Assess the effect of <i>Moringa oleifera</i> leaf extracts supplementation in preventing maternal anemia and low birth weight	Double-blind, randomized control trial study, pretest-posttest controlled	Significant increase of hemoglobin level in the intervention group ($p < 0.05$). <i>Moringa oleifera</i> extract consumption increased the hemoglobin level to 58%. In the control group, the conformity of pregnant women has no significant effect to pregnant women hemoglobin level increase. <i>Moringa oleifera</i> extract is able to retain ferritin serum level dismount up to 50%. Low birth weight was not found in pregnant women who received <i>Moringa oleifera</i> leaf extract
9	Sindhu et al./Bangalore/2013 [25]	Efficacy of <i>Moringa oleifera</i> in treating iron deficiency anemia in women of reproductive age group	The objective of studying the effect of vegetable source in the form of <i>Moringa oleifera</i> and jaggery in improvement of women suffering from iron deficiency anemia	Interventional study	The women in intervention group showed an increase in hemoglobin level. By the Student's t-test, the post-intervention data are highly significant, $t = 4.109$ ($p < 0.001$). This study shows that <i>Moringa oleifera</i> with jaggery has significantly improved hemoglobin levels of anemic women
10	Nadimin et al./Indonesia/2015 [26]	The extract of <i>Moringa</i> leaf has an equivalent effect to iron folic acid in increasing hemoglobin levels of pregnant women: a randomized control study in the coastal area of Makassar	To determine the effect of <i>Moringa</i> leaf extract for anemia prevention in pregnant women	Randomized double-blind design, pretest-posttest controlled	Hemoglobin levels of a group of <i>Moringa</i> leaf extract before intervention ($11\ 283 \pm 0777$ g/dL) increased to $11\ 754 \pm 1089$ g/dL ($p = 0.040$). Hemoglobin levels in folic iron group also increased between before and after intervention ($p = 0.002$). The amount of increase in hemoglobin concentration in folic iron group of 0.9886 ± 1.7638 g/dL higher than <i>Moringa</i> leaf extract group (0.4771 ± 1.3051 g/dL), but a large increase in hemoglobin levels between the two groups was not significant ($p = 0.168$)
11	Suzana et al./Indonesia/2017	Effect of <i>Moringa oleifera</i> leaves extract against hematology and blood biochemical value of patients with iron deficiency anemia	Investigated the efficacy of <i>Moringa oleifera</i> L. leaves to extract as an iron booster and supplement to help overcome anemia in the community	A randomized, double-blind, placebo-controlled study	The result showed that there was significantly increase of mean of hemoglobin, ferritin, mean corpuscular hemoglobin concentration, red cell distribution width, and decreased of platelets. The control groups were significantly increased of mean of the hemoglobin, erythrocytes, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and red cell distribution width. The hematocrit, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration values of <i>Moringa</i> leaves were significantly higher whereas the platelets count of <i>Moringa</i> leaves was significantly lower ($p < 0.05$) than those of control group
12	Sartika et al./Indonesia/2019 [22]	The effect of <i>Moringa</i> leaf capsule on the hemoglobin levels in young women at SMP Sabbihisma Padang	To determine the effect of leaf <i>Moringa</i> capsules on the hemoglobin levels in adolescent girls in Sabbihisma Junior High School	Quasi method of pre-test and post-test experiment with control group design	There was a significant difference in female hemoglobin levels in the case group with $p = 0.000$, while the control group was not significant with $p = 0.091$
13	Sihotang et al./Indonesia/2018 [23]	Effect of <i>Moringa (Moringa oleifera)</i> biscuit administration on hemoglobin levels of pregnant women	Analyze the effect of <i>Moringa (Moringa oleifera)</i> biscuit administration on hemoglobin levels of pregnant women.	Single-blind randomized controlled trial	Significant difference in hemoglobin levels between the treatment group and the control group after the intervention ($p \leq 0.05$). After the intervention, some respondents in the treatment group were not suffering from anemia. Administration of five pieces (60 g) of <i>Moringa</i> biscuit per day in pregnant women with anemia is recommended as one of the alternatives to overcome the undernutrition problem in pregnant women, especially to increase hemoglobin levels

Pumpkin seeds are rich in oil and protein and, given its fatty acid profile, it lies in the linoleic-oleic group such as cotton seed, corn, sesame, sunflower, and soybean oil. With high oil yield and physicochemicals characteristics similar to others commercial vegetable oil, pumpkin seed oil can be considered new and valuable source of vegetable oil [12]. Pumpkin seeds indicate highly nutritious and provide many nutrients essential for health [11]. Pumpkin seed extract contains both lignans and flavones. The results of Richter D et al (2013) research on experimental animals showed that a potential role of pumpkin seeds lignans in breast cancer prevention and/or treatment [13].

Moringa is reported to have excellent nutritional properties, low seed toxicity, high quality of oil, ability to

purify water, and adapt to poor soils and dry climates. Variation in the nutritive values depends on genetic background, environment, and cultivation methods. Leaves had high protein (28.4 g) and crude fiber (19.2 g) content but less fat (1.9 g) content whereas the fat content (0.1 g) was almost negligible in seeds. All 10 essential amino acids were present in both leaves and seeds with similar leucine, phenylalanine, threonine, and valine content. Leaves had high isoleucine and low tryptophan content. Seeds had high arginine and low valine content. Glutamate was high followed by aspartate, glycine, serine, and alanine in leaves and seeds. Roots, leaves, and seeds of *Moringa* have appreciable amino acid content. Leaves had high

calcium, potassium, sulfur, magnesium, phosphorous, and iron content whereas copper content was high in seeds. Vitamin E (tocopherol) content was high in leaves whereas seeds had high Vitamin C (ascorbic acid) content. Vitamin B2 (riboflavin) content of seeds was negligible and Vitamin E was absent. Vitamin B5 (pantothenic acid) and B9 (folate) content were similar in leaves and seeds. Folate was found in both leaves and seeds. The low content of Vitamin C in leaves may be due to oxidation loss during air drying at room temperature. It is thus evident that *Moringa* leaves are nutrient dense providing essential micronutrients. Saponins, flavonoids, steroids, glycosides, and polyphenols were present in leaves and seeds. Terpenoids were present in leaves but not in seeds. *Moringa* is known for its high antioxidant activity among all fruits and vegetables [14].

Effect of pumpkin seeds and *Moringa* leaf extract on Hb, ferritin, CRP, and outcome pregnancy

The nutritional status of pregnant women supports the birth of a healthy baby and reduces the risk of morbidity in the baby. The fulfillment of adequate nutrition during pregnancy plays a very important role in the process of fetal growth and development. The nutritional adequacy of pregnant women will affect the condition of the fetus in its growth and development during pregnancy to the outcome of birth.

Predlog promena

Pregnant women have nutrition intervention through iron supplementation, folic acid, MMN, Vitamin D, and zinc supplementation which has an impact on pregnancy outcomes. Intervention of pregnant women through pumpkin seed biscuits is expected to have an impact on Hb, ferritin, CRP levels, LBW, and reduces the incidence of stunting in children. Pumpkin seeds are a good source of phytoestrogens and may exert week estrogenic or anti-estrogenic effects. Studies in postmenopausal women have demonstrated that pumpkin seed oil, at a dose of 2 g per day for 12 weeks, can decrease blood pressure and reduce menopausal symptoms also decrease CRP [8]. Intervention with ready-to-eat cereal (providing 7.1 mg iron/day) plus 30 g of pumpkin seed kernels (providing 4.0 mg iron/day) for 4 weeks contributes to maintaining optimal nutritional status and minimizing the likelihood of iron insufficiencies in woman reproductive age which was increasing of serum iron, percent transferrin saturation, and reducing TIBC [15]. The formulation of amla, jaggery, and pumpkin leaves shows a greater potential in the combination therapy of herbal medicines in the management of anemia which was influence to increase hemoglobin, Vitamin C, and iron plasma in adolescents girls with iron deficiency anemia [16]. Study in Egypt was showed that

supplementation of cooked soybean, cooked lentils, sesame seed, pumpkin seed, molasses, and mixture of them (soybean + lentils + sesame seed + Pumpkin seed powder + molasses) with ascorbic acid in rats revealed significant increase in Hb, Hct, mean corpuscular volume, mean corpuscular Hb, MCH concentration (MCHC), red blood cell, white blood cell, serum iron, and significant decreased in PT and TIBC [4]. Treatment of atherogenic rats with pumpkin seeds significantly decreased serum concentrations of total cholesterol and low-density lipoprotein-cholesterol, which explained pumpkin seeds supplementation has a protective effect against atherogenic [17]. Pumpkin seed oil extracted from *C. maxima* has the potential for prevention or treatment of urinary disorders including dysfunction urinary tract on human overactive bladder [18]. Study supplementation pumpkin seeds biscuit to combat malnutrition in Pakistan was showed that pumpkin seed flour can be supplemented successfully to partially replace wheat flour to prepare highly nutritious biscuits without affecting its overall acceptability [19].

Intervention of pregnant women through *Moringa* leaf extract capsules is expected to have an impact on Hb, ferritin, CRP levels, LBW, and reduces the incidence of stunting in children. Supplementation of *M. oleifera* leaf extracts significant increases of Hb level to 58% compared with the control group, *M. oleifera* extract is able to retain ferritin serum level dismount up to 50% [20]. Intervention of *M. oleifera* L. leaves significantly increased of mean Hb, ferritin, MCHC, and red cell distribution width and decreased of platelets. One thousand and four hundred milligrams of *Moringa* extract per day increased significantly hemoglobin and the result higher than control group [21]. *Moringa* leaf capsule can increase Hb level of adolescent girl, especially for anemia [22]. Supplementation of *M. oleifera* biscuit significant influences the increasing of hemoglobin in pregnancy anemia [23].

Moringa extract give evidence to utilize local food in order to prevent anemia and adverse pregnancy outcome [24]. *M. oleifera* has significantly increased hemoglobin concentration among anemic women [25]. Intervention of *Moringa* leaf significant increasing Hb levels of pregnant women [26]. For birth weight, supplementation using *Moringa* extract has positive effect on LBW prevention [20].

Conclusion

The provision of pumpkin seed biscuits and *Moringa* leaf extract to pregnant women can affect Hb, ferritin, and CRP levels and can affect maternal nutritional status and affect pregnancy outcomes.

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