Effect of *Moringa Oleifera* Leaf Extracts Supplementation in Preventing Maternal DNA Damage

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Abstract-The extracts of *Moringa oleifera* leaf have been proven to have potent anti oxidant activity, prevent oxidative damage and afford significant protection against oxidative damage. This study aims to assess the effect of *Moringa oleifera* leaf extracts supplementation in preventing maternal oxidative DNA damage. This was a double bind, randomized control trial study, which conducted in Gowa District, South Sulawesi Province, Indonesia, from January to December 2012. Seventy six of first trimester pregnant women enrolled in this study. They were randomized equally to the intervention and control group. Intervention group received *Moringa oleifera* leaf extracts with iron folic acid capsules, while the control group received only iron folic acid capsules for three months. Before and after intervention, blood samples were extracted for measuring 8-hydroxy-2' -deoxyguanosine (8-OHdG) using enzyme-linked immune sorbent assay (ELISA). The effect of intervention was analyzed by Independent T test and paired T test. In the supplemented group, mean concentration of 8-OHdG was 3616 pg/ml and significantly reduced during the intervention by 36% (p=0.000). Levels of 8-OHdG were higher in the control group 3690pg/ml, and reduced by 30.4% (p=0.019). The mean difference was not significant between two groups (p= 0.278). The result indicate there was no significant difference in the level of 8-OHdG between intervention and control group but the percentage of velocity in the intervention group larger than control group. Further research is needed to be implemented on non pregnant women, so the control group could receive pure placebo.

Index Terms-*Moringa oleifera*, maternal DNA damage

I. INTRODUCTION

Diet in pregnancy and the supply of nutrition to the development of the embryo or fetus is one of the important environmental factors that affect fetal growth, cell proliferation, and DNA replication in the critical period of life. Nutrition is very important to maintain the integrity of the genome because of its role as an enzyme cofactor or as part of a protein that plays a role in DNA synthesis and repair, prevention of DNA damage due to oxidative stress reactions, and maintain DNA methylation[1].

DNA damage, especially that caused by oxidative stress, has been shown to be associated with abnormal pregnancy outcome including pre-eclampsia (PE) and intrauterine growth restriction (IUGR)[2,3]. The results showed that DNA damage was significantly associated with pregnancy develop into PE and IUGR[4].

DNA damage can be caused by micronutrients deficiency. Micronutrients have an important role in protecting DNA damage by providing a co-factor required for the proper functioning of enzymes that play a role in DNA repair. Fenech (2005)[5]has identified that a low intake of calcium, folate, nicotinamide acid, vitamin E, retinol, beta-carotene and significantly associated with genomic instability. Zinc has also been shown to play an important role in the repair of DNA damage[6].

DNA damage in pregnant women, as measured by the concentration 8-OHdG has been shown to be associated with PE and IUGR. The mechanism is still not clear but it is thought because of ischemia or hypoxia of the placenta. Injury due to ischemia may contribute to the oxidative stress and cause the release of oxidative stress to the maternal and placental circulation is likely to result in DNA damage and could be the basis of impaired fetal growth and development[7].

Anti-oxidants such as vitamins A, and E can prevent 8-OHdG production due to anti-oxidant activity in inhibiting free radicals[8]. Thomas P (2009)[9]in a review also reported that supplementation with antioxidant vitamins and B vitamins can reduce the frequency of micronuclei (a marker of DNA damage). An intervention study found that the frequency micronuclei significantly associated with serum levels of vitamin B12[10]. Smolkova (2004)[11] reported that supplementation with antioxidants had a significant effect in reducing the amount of 39% micronuclei.

Oxidative DNA damage can be measured with 8-OHdG. 8-OHdG is one of the mosteasily formed oxidative DNA lesions. 8-OHdG is a modified nucleoside base, which is very often studied and detected as DNA damage products excreted in the urine when DNA damage occurs[12]. Examination of DNA damage using ELISA is a rapid and sensitive immunoassay for the detection compete with the amount of 8-OHdG in urine samples, serum, or saliva[13].

Moringa oleifera leaves have long been used to overcome the problem of malnutrition among children, pregnant women, and breastfeeding[14]. In addition, antioxidant activity of *Moringa* leaf have been investigated. Sreelatha and Padma

(2009)[15] reported that 1 g of extract of *Moringa* leaf contain non enzyme anti-oxidants such as ascorbic acid 6.6 mg, tocopherol 6.53 mg, and carotenoids 92.38 mg. Research on antioxidant enzymes in extracts of *Moringa* leaf also showed a high rate activity of superoxide dismutase (SOD), and catalase (CAT). Similarly to the phenolic (45.81 mg/g) and flavonoids (27mg/gr). Since polyphenols act as an anti-oxidant that is a high number indicates that extracts of *Moringa* leaf contain high antioxidant activity. This study further reported antioxidant activity in preventing DNA damage. By measuring the activity of λ DNA (72.45 ug / ml) of *Moringa* leaf extract can significantly reduce DNA damage induced by H2O2 oxidant.

With micronutrients substances and high anti-oxidant compounds, the extracts of *Moringa* leaves can be used as an alternative supplement for pregnant women to prevent DNA damage. Prevention of DNA damage can decrease the incidence of pregnancy complications such as PE and IUGR and which lead to the normal birth weight baby[16]. The purpose of this study was to assess the effect of extracts of *Moringa Oleifera* to the prevention of DNA damage in pregnant women.

II. MATERIAL AND METHOD

Study design was double blind, randomized, control trial. First trimester pregnant women who tested positive by dipstick pregnancy (urine test) that meet the inclusion and exclusion criteria were recruited consecutively. Totally, 76 pregnant women who enrolled, randomized to group who received *Moringa* leaf extract capsules with iron 60 mg and folic acid 400µg (intervention) and iron 60 mg with folic acid 400 µg capsules (control). Capsules were given for three months. Before and after intervention blood samples were extracted for measuring 8-hydroxy-2' -deoxyguanosine (8-OHdG).

Moringa leaves were drawn from the *Moringa* tree that growing in Makassar and Majene district. Moringa leaf washed by dipping it into water ways and douse with water several times. After being washed, dried *Moringa* leaf-aired aerate for 2 hours, and then dried in a heating clothesline using incandescent lamps with a temperature of 38^0C for 2 x 24 hours. The dried leaves were kneaded by hand until a small shielded. *Moringa* leaves that already half smooth then taken to the laboratory. *Moringa* leaves were taken and there are 9 g plus 1 g bitter macerated with methanol to within 1 x 24 hours, this treatment was repeated 3 times, then filtered to separate and extract the pulp. The extract were evaporated at a temperature of 600^0C for 2 x 24 hours. The result was dried frozen (freeze dryer) for 1 x 24 hours. The pulp was dried at a temperature of 400^0C for 1x24 hours. The results of 200 mg of extract was mixed with 800 g of the residue, and then inserted into an extract *Moringa* capsules weighing 1 g.

Examination of 8OHdG performed in accordance with protocol 8-hydroxy 2 deoxyguanosine ELISA Kit Abcam production of (101245)[17]. Plate, buffer solutions, standards, antibody, and reagent issued to room temperature before done. Plasma samples of homogenized with Fortex. Plate prepared, then added reagent such as buffer, standard, sample, and antibody number 50 ml each corresponding to wells that have been specified in the protocol. Plates were then incubated for 18 hours at a temperature of 20^0 C. Wells then emptied and rinsed five times with wash buffer. Then added 200 ml Ellman's reagent to each well and 5 ml of tracer to the total activity well. Plate and then covered with plastic films and shaken with an orbital shaker for 90 minutes. After the plate was read by the machine wiped and read with Elisa reader at a wave length of 420 nm.

Data were analyzed using statistical package for social science (SPSS). The mean of 8-OHdG levels before and after intervention were analyzed using paired T test, and the mean between the two groups using independent T test.

III. RESULTS AND DISCUSSION

8-OHdG levels in the intervention and the control groups can be seen in Table 1. below.

Table 1. Differences 8-OHdG Levels at the Beginning and the End, as well as the Inter-Group Differences

Group	Level of 8-OHdG Beginning (pg/ml)	Level of 8-OHdG End (pg/ml)	P-Value
Intervention	5684	3616	0.000
Control	5307	3690	0.019
P-Value	0.596	0.278	

The decrease in 8OHdG levels was significant when compared to the time before and after the intervention. Nevertheless, when compared between the intervention and control groups, no significant differences appear. Although the analysis of the two groups did not give a significant difference, but if we observe a decrease in the percentage rate of 8 OHdG levels, the intervention group gave greater rate as we can see at Table 2. This result confirmed studies on green tea and red wine that had beneficial effect to reduce 8-oxodG concentrations[18,19].

Table 2. Percentage of Velocity of 8-OHdG Before and After

Group	Mean of 8-OHdG Before (pg/ml)	Mean of 8-OHdG After (pg/ml)	Velocity (%)
Intervention	5684	3616	36,3 %
Control	5307	3690	30,4 %

DNA damage in this study was determined by ELISA method that uses 8-OHdG as a biomarker. Deoxyguanosine (dG)

is one of the constituent DNA and when oxidized will turn into 8 hydroxy-guanosine (8-OHdG). In addition to undergo oxidation, hydroxylation of guanosine can also be experienced as a normal metabolic response or due to other environmental factors. Increased levels of 8-OHdG associated with aging and pathological disorders, including depression, cancer, diabetes, hypertension, and oxidative stress[20]. Supplementation with vitamins and minerals with antioxidants has been shown to prevent damage to DNA[9].

In this study, administration of *Moringa* leaf extract that has been proven to contain vitamins and minerals as well as anti-oxidant enzymes are expected to prevent DNA damage[21]. Dietaryantioxidants areexpected to inhibit the formation of oxidative DNA damage. WhenROS are formed in vitro or by oxidative stress in vivo, severaltypes of oxidative DNA lesions are formed, including small baselesions and exocyclic adducts[20]. The results showed that 8-OHdG levels decreased significantly before and after the intervention, both in the intervention group ($p = 0.000$) and in the control group ($p = 0.019$). Significant changes in both groups may be caused by these two groups therefore get a supplement containing folate and iron, two micronutrients that play a role in DNA synthesis and oxidative DNA damage repair [22,23].

Independent T test on the difference between the intervention and control groups was not significant ($p = 0278$). However, when viewed from the rate of decrease (velocity) intervention group had a rate of decline in the percentage in the intervention group (36.3%) greater than the control group (30.4%). This is possible because in the intervention group, *Moringa* leaf extract contains anti-oxidants that much faster can prevent oxidative stress plays a role in DNA damage due to oxidation. In this study, a control group given a placebo cannot be pure, because in pregnant women in Indonesia, iron and folic acid supplementation has become a national program. This can affect the results of the difference decreased levels of 8-OHdG in both groups.It is paralel with Moller's statement in a review, that consumption of multiple vitamins can not provides better protection against oxidative DNA damage than does single-vitamin supplementation[24].

In conclusion,Moringa Oleifera leaf extracts have no significant effect to reduce DNA damage compared to the iron folic acid. Further research is needed to proof their effect on the people with the normal condition or non pregnant, so the control group could be given pure placebo.

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