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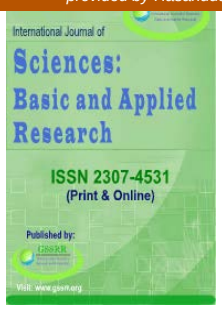

Effect of Honey and *Moringa Oleifera* Leaf Extracts Supplementation for Preventing DNA Damage in Passive Smoking Pregnancy

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Abstract

Honey and *Moringa* leaf extract was known to have potent antioxidant activity that can prevent oxidative stress and DNA damage and repair hematologic status including in pregnant women who exposed to smoke in their environment. The purpose of this study was to know the effect of providing natural antioxidants (honey + *Moringa* leaf extract) against oxidative stress and DNA damage in pregnant women who become passive smokers. This study used a non-randomized group pre-post test design with a sample of passive smokers are pregnant women who live in Takalar regency, province of South Sulawesi. 80 samples were third trimester pregnant women who participated in the study and selected by purposive sampling. The samples were divided into two treatment groups ie groups MK who consume honey + *Moringa* leaf extract and K groups that only consume *Moringa* leaf extract in 90 days. Before and after the intervention both groups were measured Malondealdehyde (MDA) and 8-hydroxy-2'deoxyguanosine (8-OHdG) using the ELISA test.

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The results showed decreased levels of MDA in the treatment group honey + *Moringa* (KM) (1.84±20.03 nmol/ml, p>0.05), while the treatment group *Moringa* leaf (K) increase (0,22±15.30 nmol/ml, p>0.05) and there was a significant difference between the two groups (p <0.05) levels of 8-OHdG in the treatment group honey + *Moringa* (MK) decreased significantly (6:09 ± 31.89 ng / ml, p <0.05), whereas in the group of *Moringa* (K)show a significant increase (6.87 ± 29.41ng / ml, p <0.05), and there was a significant difference between the two groups (p <0.05). There was the effect of honey and *Moringa* leaf extract on the prevention of DNA damage in pregnant women passive smokers. Further research on the effective dose of honey and extract of *Moringa* leaves and the long giving effectively preventing DNA damage, the further research on the exact gestational age of pregnant women consume honey and *Moringa* leaf extract effectively for preventing DNA damage.

***Keywords**:* pregnant women; passive smokers; honey; *Moringa* leaf extract; DNA damage.

1. Introduction

The current epidemiological studies have found a link maternal smoking and other environmental exposures have increased the risk of low birth weight, premature birth, spontaneous abortion and inhibited fetal growth. Cigarette smoke was one source of free radicals, the number one suction entered were 10^{14} molecules free radical oxidants produced by cigarette smoke. The resulting oxidant and high H2O2 content in cigarette smoke will ease free radical propagation [1].

Pregnant women who are exposed to cigarette smoke and smoking status (passive, active) tend to have a smaller size of the placenta, indicating a disturbance in growth placenta [2]. Presence of cigarette smoke exposure during pregnancy can cause effects on an increase in serum thiocyanate concentration and the effect on weight placenta [3]. According to Surinah, Carbonmonoksida (CO) inhaled by pregnant women will carry over into the mother's bloodstream, causing the baby and placenta acceptance oxygen was reduced, which also means to reduce nutritional on fetus [4]. The further contagion placenta will further expand in the area of the uterus to look at the surface area of the uterus to meet the need of oxygen and nutrients, which resulted in the placenta will be thinner. This was consistent with research of Bekti (2011), oxidative stress on placenta and circulation system will be dysfunction and endotel cell damage [5].

Excessive formation of ROS caused by exposure to toxic agents or insufficiency of various defense mechanisms that generate oxidative stress injury in cellular membranes, DNA and proteins. Index of oxidative damage to DNA and lipids has been used as a substitute for genotoxicity biomarkers for assessing the carcinogenic potential of environmental pollution. 8- hydroxide-oxyguanosine (8-OH-dG) was pretty much, and was easily detectable as a product of oxidative damage to DNA, and regarded as a relevant biomarker of cellular oxidative stress. Malondialdehyde (MDA) was lipid peroxidation biomarker, have also been used to assess the biochemical endpoints in response to oxidative stress [3].

Some micro-nutrients (vitamins and minerals) was necessary as an enzyme cofactor or as part of the protein structure (metalloenzymes) that play a role in the synthesis and repair of DNA, DNA oxidative damage prevention and maintenance of DNA methylation [6]. Iron (Fe) and other micro-nutrients (Zn, Se, folic acid,

vitamin C, vitamin E, vitamin A) has a very important role in the maintenance of DNA. Iron (Fe) produces antioxidant enzymes and a number of enzymes required for the metabolism of nucleic acids, and enzymes for the synthesis and repair of DNA [7, 8]. Lack of iron can damage biological pathways and cause oxidative stress, cell death, genomic instability and isncreases the risk of cancer [7,8].

Micro-nutrients have been shown to affect fertility, embryogenesis and placentation. Micronutrient supplementation has been widely used for the prevention of disorders in pregnancy, including as a result of environmental pollution (exposure to cigarette smoke) that can cause oxidative stress [9].

One of the potential of local materials that are rich in micronutrients and widely available but not fully utilized by the community, including a group of pregnant women were honey and *Moringa* leaves. These food items are easily found throughout Indonesia and its utilization by the community traditionally has been used empirically. Antioxidant activity of honey was generally associated with phenolic compounds and flavanoid [10]. Honey given at therapeutic doses tended without prooxidant and can produce effect of antioxidants synergistic. Honey can improve oxidative stress by free radicals attack OONO-O_2 and non-free radicals such as NO [10]. Recent studies show that honey repaire oxidative stress by regulating Nrf2, a transcription factor important intracellular. The evidence also suggests that honey may reduce inflammation as evidenced by the inhibition of the production of NO and prostaglandin E. Besides that honey research has also proved the implication of oxidative stress and inflammation in the pathogenesis and complications of the disease diabetes mellitus and hypertension [10].

In vitro studies found that the extract of *Moringa* leaves contain antioxidant compounds non enzymatic strong and can clean up free radicals,giving protection against oxidative DNA damage. *Moringa* leaf extract contain polyphenolic compounds, flavonoids and phenols as antioxidant components which become free radical scavenging [13]. Based on the results of research utilization of *Moringa* leaf extract to the number of erythrocytes in Wistar rats were exposed to cigarette smoke are found there was an increase in the number of erythrocytes in provision of extract *Moringa* leaves 100-200 mg.kg / day compared with no provision of *Moringa* leaf extract and a dose of 400 mg / kg / day [10]. In addition, other studies regarding the use of *Moringa* leaf extract on hepatic MDA levels in Wistar rats were exposed to acute cigarette smoke are found there are significant provision of *Moringa* leaf extract 400 mg / kg as an antioxidant to decrease hepatic MDA levels in rats exposed to acute cigarette smoke [11]. Research conducted by Luqman S, et. al, (2012) shows that there are increased levels of hemoglobin in rats which given *Moringa* leaf extract 100 mg / kg BW [12].

The publications about the utilization of *Moringa* leaf extract in pregnant women was good for the prevention of anemia and low birth weight as well as the prevention of DNA damage have been found but there were never found about the exposure to environmental cigarette smoke. Besides of that, there are rarely about the publications about the use of honey as a source of antioxidants in pregnant women. The existed publications never been reported on the effect of honey and *Moringa* leaf extract together in the prevention of impaired fetal growth caused by environmental tobacco smoke exposure in the third trimester pregnant women. Based on this it is important to make further study about the role of honey and *Moringa* leaf extract as a source of natural antioxidants in preventing the negative effects of cigarette smoke on the environment of pregnant women

against DNA damage and response placental vascular contractility which may have an impact on fetal growth disorders (fetal hypoxemia) and eventually resulting in low birth weight (LBW).

This study aims to determine the effect of natural antioxidants (honey + *Moringa* leaf extract) against oxidative stress and DNA damage in pregnant women passive smokers. In the event of a decrease in the levels of MDA and 8-OHdG in pregnant women passive smokers who consume honey and *Moringa* leaf extract, it can give input and suggestion for nutrition practitioners to prevent the effects of free radicals caused by smoking with honey and *Moringa* leaves.

2. Material and Method

This type of study was an experiment research (Quasy Eksperiment). With Non-randomized study Group pre - Post test design. At design consists of MK treatment groups (Honey + *Moringa*) and K treatment group (*Moringa*). The number of samples in each group were at least 40 people, so it takes 80 pregnant women passive smokers into 2 groups. After accomplished with the possibility of a 10% drop out rate, the total study subjects were 88 pregnant women passive smokers

All pregnant women passive smoker who meet the criteria were divided into two groups by simple random sampling. The first group was a group of pregnant women passive smoking who received supplements honey (40 mg / day) and *Moringa* leaf extract 1000 mg / day. The second group is passive smoking pregnant women who only received the extract of *Moringa* (1000 mg / day). Before and after the intervention carried out inspection of MDA and 8-OHdG. Honey was used in this study was pure honey from apis milifera, standardized and safe for pregnant women with a dose of 40 mg / day in 2 doses *Moringa* leaves are selected in this study came from Gowa and Takalar.

Moringa leaves that have been picked and then washed, drained for 2 hours, then threshed in order to separate from the stalk. Furthermore, *Moringa* leaf was dried using an oven with a temperature of 30-40 ° C for 3-4 hours or until dry to a moisture content <10%, the process of maceration were: the dried *Moringa* leaves soaked with 30% ethanol for 1x 24 hours. Then squeezed and filtered using gauze to separate and extract the dregs. The extract was evaporated to eliminate the ethanol for 48 hours at a temperature of 30-40 ° C, freeze dried in a freeze dryer for 24 hours. Grinding done to obtain flour extract. Furthermore, the quality control was done through the examination of water content, microbial, and organoleptic. Then mix the dry extract + *Moringa* powder with a ratio of 4: 1, then made capsule filling. Each capsule of 400 mg + 100 mg extract of *Moringa* flour. The dose given 1000 mg / day in 2 doses.

The level of MDA and 8-OHdG by ELISA of Bioassay Technology Laboratory. The checking carried out in the Laboratory Hospital of Hasanuddin University. Plate, standard solution, standard antibody, and biotin, Elisa solution, chromogen solution A and B, were stored at room temperature before using. Serum samples were stored at room temperature 10-20 minutes, then centrifuge (2000-3000 rpm) for 20 minutes. Adding a label antibody, biotin and Elisa solution on serum samples and standards simultaneously. Washing the plate 5 times, then adding chomogen solution A and B. Incubation for 10 minutes at temperatures of 37 ° C, adding the stop

solution, then reading the OD value within 10 minutes.

Data were analyzed using SPSS version 22. The two-sample t test or Wilcoxon test relates to examine differences in the levels of MDA, the concentration of 8-OHdG, before and after the intervention in each intervention group. To see the changes mean difference between the two groups used t test (independent t test) or Mann Whitney U-test.

3. Results and Discussion

3.1 The characteristic of study subject

Table 1 shows pregnant women in both groups are passive smokers with differences in exposure in the home was same (80%) and the more MK group as moderate smokers (80%) while in Group K as light smokers (75%).

Table1: The Descriptive analysis variables of study based on treatment groups

Variables	**MK Treatment n= 40**	**K Treatment n=40**	**P value**
Smoke exposure at home :			
Yes	32(80%)	33 (82.5%)	0.00
No	8 (20%)	7 (17,5%)	
The cigarettes consumption a day:			
The light smokers	5 (12.5%)	30(75%)	0.00
The moderate smokers	32(80%)	8 (20%)	
The heavy smokers	3 (7.5%)	2 (5%)	
MDA			
Mean ± SD	26.30±12.21	15.64±13.05	0.00
8-OHdG			
Mean ± SD	47.62±54.99	38.56±49.39	0.44

Source: primary data 2015

MDA levels in the group of honey + *Moringa* (MK) show the average decrease insignificantly before and after the intervention (1.84±20.03 nmol/ml, p>0.05). whereas *Moringa* group (K) show average increase insignificantly before and after the intervention (0:22 ± 15:30 nmol / ml, p> 0.05)). But there were significant differences between the two treatment groups (p <0.05) (Table 2)

Tabel 2: The results of the difference in average levels of malodealdehyde(MDA) pretest and posttest for both treatment groups

Malondealdehyde (MDA)nmol/ml	**Pretest** **X±SD**	**Postest** **X±SD**	**P value**	**Δ(T3-T0)** **X±SD**	**P value**
Honey + *Moringa* (MK)	26.30±12.21	24.45±14.22	0.09	1.84±20.03	0.00
***Moringa* (K)**	15.64±13.05	15.86±10.97	0.23	0.22±15.30	

Source: primary data 2015

The levels of 8-OHdG in the group Honey + *Moringa* (MK) on average has decreased significantly before and after the intervention (6:09 ± 31.89ng / ml, p <0.05). whereas *Moringa* group (K) on average has increased significantly before and after the intervention (6.87 ± 29.41ng / ml, p <0.05) There were significant differences between the two treatment groups (p <0.05) (Table 2).

Table 3: The results of the difference in average levels of 8OHdG pretest-posttest for both treatment groups

8 OhdG(ng/ml)	**Pretest** **X±SD**	**Postest** **X±SD**	**P value**	**Δ(T3-T0)** **X±SD**	**p value**
Honey + *Moringa* (MK)	47.62±54.99	41.53±50.05	0.02	6.09±31.89	0.00
***Moringa* (K)**	38.56±49.39	45.43±13.50	0.04	6.87±29.41	

Source: primary data 2015

Pregnancy was an inflammatory process can result increased susceptibility to oxidative stress [14]. Increased oxidative stress can trigger free radical attack on important molecules such as fats, proteins, including enzymes and DNA [15]. The inflammatory process associated with reactive oxygen species (ROS) and nitrogen species oxidative (NOS) which can cause single and double strand breaks (SSB / DSB) DNA [16]. The ability of repair DNA has been proven to decrease in pregnant women, making them more sensitive to endogenous and environmental toxins including environmental cigarette smoke can cause disease [17]. Provision antioxidants in the form of supplements or foods containing antioxidants (such as honey and *Moringa* leaf extract) were very

important to increase antioxidant levels in pregnant women and prevent pregnancy complications associated with oxidative stress [18, 19].

The results of this study found that the average levels of 8-OHdG passive smoking pregnant women who received honey + *Moringa* leaf extract (MK) pre-post test sequentially were 47.61 ± 54.99ng / ml and 41.53 ± 50.05 ng / ml. while those who only get *Moringa* leaf extract (K) average levels of 8-OHdG pre-post test sequentially were 38.55 ± 49.39ng / ml and 13:50 ± 45.43 ng / ml. The average level of 8 - OHdG in both groups was higher than the average levels of 8-OHdG group of pregnant women who received the extract of leaves of *Moringa* on research of [20] were 14.13 ± 1.22 nmol / ml and 14.28 ± 1, 64 nmol / ml. The difference was probably due to differences in the sample used in the study by [20] using the urine while on this study using serum as well as the role of environmental smoke exposure of pregnant women.

The statistical analysis levels of 8-OHdG group receiving honey + *Moringa* leaf extract (MK) showed significant differences compared to the group *Moringa* (K) ($p < 0.05$). This study found that a decline in the group given honey + *Moringa* leaf extract (MK) (6:09 ± 31.89ng / ml) compared with the group given *Moringa* leaf extract (K) increased (6.87 ± 29.41ng / ml). This shows that the provision of natural antioxidants (honey + *Moringa* leaf extract), especially in the third trimester of pregnancy can prevent DNA damage that was characterized by reduced levels of 8-OHdG in the group given honey + *Moringa* leaf extract. While research of [20] also found that provision of only *Moringa* leaf extract can not reduce levels of 8-OHdG (an increase of 0.49 ± 2.18 ng / ml). In this study also found that honey + *Moringa* leaves have better skills in the prevention of DNA damage in pregnant women passive smokers than only provision *Moringa* leaf extract.

4. Conclusion.

There was the effect of honey and *Moringa* leaf extract on the prevention of DNA damage in pregnant women passive smokers. There was conducted further research on the effective dose of honey and extract of *Moringa* leaves and longer provision to effectively prevent DNA damage and further research on the exact gestational age of pregnant women consume honey and *Moringa* leaf extract that was effectively for preventing DNA damage and the further research on the benefits of honey and leaves of *Moringa* as free-radical scavengers, especially for active smokers.

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