

Iron-Folate Supplementation during Pregnancy for Prevent Oxidative Stress in Pregnant Rats : Level of MDA, Creatinine, Glucose, Erythrocyte, Blood Pressure, Body Weight and Number of Offspring

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History

- Submission Date: 05-11-2019;
- Review completed: 19-11-2019;
- Accepted Date: 27-11-2019.

DOI : 10.5530/pj.2020.12.28

Article Available online

<http://www.phcogj.com/v12/i1>

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ABSTRACT

Background: Iron and folic acid deficiency during pregnancy can increase oxidative stress and result in impaired intra-uterine growth, abortion and preeclampsia. Folate is trace nutrient that influential for essential role for epigenetic mechanism cues into changes in gene expression and had impact health development. This study aimed to determine the effect of several doses of iron and folate supplementation on level of: MDA, glucose, creatinine, maternal body weight and number and birth weight of offspring. **Methods:** This research was conducted in the laboratory of the Center for Food and Nutrition Studies, Gadjah Mada University Yogyakarta. Experimental research with posttest only control group design with a number of samples: 20 pregnant rats, divided randomly into 4 groups. The control group (C) was given standard feed (AIN-93G), KI: added iron 1,8 mg/200gBW and folic acid 0,0023mg/200gBW, KII: added iron 3,6 mg/200gBW and folic acid 0,0045 mg/200gBW, KIII : added iron 5,4mg/200gBW and folic acid 0,0068 mg/200gBW. Duration of treatment 20 days. Measurement of body weight, blood pressure and then taken blood samples at the 21st day for examination of MDA, glucose, creatinine, erythrocyte level. Sectio caesarean to performed the number and body weight of offspring. Data obtained were analyzed using one way Anova followed by Post hoc LSD. **Results:** there are significant different ($p < 0.001$). on level of : MDA, glucose, creatinine, maternal body weight, average number and fetal weight of offspring between treatment group compare to control group. **Conclusion :** Iron and folate supplementation during pregnancy can decreased level of oxidative stress and better pregnant outcome.

Key words: Pregnancy, Iron, Folic acid, MDA, Oxidative stress.

INTRODUCTION

Pregnancy is a critical period full of physical and psychological changes related to embryonic development as a result of fertilization to become a fetus in the uterus, marked by fetal growth, maternal adaptation, preparation for child birth and breast feeding.¹ Period of plasticity whereby fetal development may be significantly influenced by environmental factors, such as maternal nutrients, like the inherited genetic profile. Epigenetics has been found to play a role in fetal programming to the changes an individual's genetic code that can alter gene expression, without changing the DNA sequence. Effectively controlling environmental factor needed to successfully genes expression that can enable the developing fetus.² Low grade of free radicals are needed in the right amount for the continuous of cell proliferation and differentiation processes, excessive amounts can cause oxidative stress that cause cell damage included: DNA, lipids and proteins which is actually harmful to the continuity of pregnancy such as abortion, IUGR and Preeclampsia. Iron and folate deficiency play a role in the formation of excess free radicals, but excess iron also risks increasing free radicals.

Superoxide radicals are a form of free radicals that occur a lot in normal pregnancy, but in a state of hypoxia the concentration of superoxide ions increases. Compared to normal pregnancy oxidative stress and proinflammatory markers are higher in preeclampsia.^{1,3,4} Increased lipid peroxidation index in the placenta of women with preeclampsia and in the serum and plasma of women with IUGR fetuses.⁵

Studies in rats have shown that dietary iron elimination can cause tissue damage through oxidative stress and mitochondrial damage. Cellular iron homeostasis plays an important role in mitochondrial biogenesis, and iron deprived cells show downregulation of mitochondrial protein levels and oxidative capacity. The availability of an adequate supply of nutrients may be the most important environmental factor influencing pregnancy health outcome.⁶

In the skeletal muscle showed that interactions between genetic (SNP), epigenetic (DNA methylation), and non-genetic (age) factors influence gene expression and metabolism. Prenatal factors including mode of conception, maternal smoking, and maternal diet have indeed shown to effect the DNA methylation pattern.⁷ Biological activity folate together with vitamin B12 plays a crucial role

Cite this article: Wahyuwibowo J, Aziz A, Safitri E, Minidian F, Zulaikhah ST. Iron-Folate Supplementation during Pregnancy for Prevent Oxidative Stress in Pregnant Rats: Level of MDA, Creatinine, Glucose, Erythrocyte, Blood Pressure, Body Weight and Number of Offspring. Pharmacogn J. 2020;12(1):186-91.

in the one-carbon metabolism and embryonic development. In this context, low dietary intakes of folate are associated with various clinical symptoms, especially neurological and developmental disorders.⁸ Many factors during pregnancy can impact the child's epigenetic status, including the health of the mother. A study carried out in the UK identified particular locations and CpG sites within the genome, where methylation patterns of the offspring were altered by mothers' gestational diabetes status. Maternal weight during pregnancy was also associated with altered methylation patterns in the child's DNA and later infant adiposity.²

The nutritional status of the maternal during the pregnancy is a critical period, where the status of a good nutritional is an important factor that influences the health of the mother and child. Pregnant women must be able to meet nutritional needs for themselves and the fetus they contain. The risk of pregnancy and childbirth complications is lowest if the weight gain before giving birth is adequate⁹ Pregnant women with Chronic Energy Deficiency increases the risk of low birth weight.¹⁰ Epidemiological studies and experimental studies in animals show an association between low birth weight and increased incidence of degenerative diseases such as hypertension, type II diabetes, metabolic syndrome, insuline resistance, and obesity. Babies who born with more weight are also at risk of developing metabolic syndrome as adults.⁵

FOLIC ACID METABOLISM

Folic acid is a synthetic form of vitamin B9 which is a very important part in the conversion of homocysteine to methionine so that folic acid deficiency results in an increase in Hcy Folic acid is inactive and has no biological activity before it is activated by the enzyme methylene tetrahydrofolatereductase (MTHFR) converted to tetrahydrofolate acid by the dihydrofolate reductase enzyme, then by endocytosis mediated by receptors entering cells to maintain normal erythropoiesis, changes in amino acids, methylation tRNA, formate formation and use, synthesis of purine and thymidilate nucleic acid. Together with vitamin B12 as a folic acid cofactor regulates homocysteine levels (Hcy) through Hcy remethylation to methionine mediated by the methionine synthetase enzyme. Folate deficiency will be increase Hcy level, is a risk factor for CKD progression and cardiovascular complications. Tetrahydrofolate derivatives are used in two denovo stages purine biosynthesis. Folate deficiency causes disruption of purine synthesis, as a result there is a disruption of cell division and differentiation. Purine along with the pyrimidine base forms a helix DNA arrangement, so folate deficiency can also result in disruption of DNA synthesis and cause various body problems such as cancer and immune function disorders.¹¹⁻¹⁴ Hcy contains a thiol group that is very reactive and quickly undergoes autoxidation in the presence of oxygen, Fe and Cu ions to produce a potential ROS consisting of superoxide anion, H₂O₂, and hydroxyl radicals indicating that Hcy autoxidation is one form of ROS. Hcy directly activates NADPH oxidase to produce superoxide anions and also activated the phosphorylation of the cytosolic p47phox subunit

through activation of C-β protein kinase which increases the formation of oxidase enzymes. Hcy increased the levels of mRNA from NADPH oxidase together with increased production of ROS in endothelial cells. Hcy also increases NOX-4 and causes translocation into mitochondria. Therapy with folic acid administration to reduce Hcy returns the level of methylation DNA to normal and improves gene expression.^{11,15} Alterations to folic acid status could directly impact the amount and patterns of DNA methylation. Specifically, methyl donor nutrients such as methionine, folate, betaine, and choline have been implicated in alterations in methylation patterns. These nutrients are directly related to one-carbon metabolism, the process that generates SAM, the major substrate for DNA methylation.¹⁶

RESEARCH METHODS

This research has received Ethical Clearance from the Ethical Commission of the Faculty of Medicine, Sultan Agung Islamic University, Semarang with numbers: 317 / XII / 2016 / Bioetic Commission. This experimental study with a post test only control group design uses 20 pregnant rats, divided into 4 groups randomly. The control group was fed AIN-93G only, KI was added iron 1,8 mg/200 gBW and folic acid 0,0023 mg/200gBW, KII added iron 3,6 mg/200g BW and folic acid 0,0045 mg/200gBW, KIII added iron 5,4 mg/200gBB and folic acid 0.0068 mg/gBW. The doses equivalent with 1 tablet of iron-folate supplementation that were usually given for pregnant women in Indonesia containing 200 mg of ferrous sulfate (equivalent to 60 mg of elemental iron) and 0.4 mg of folic acid.¹⁷ The treatment was carried out for 20 days. That were corresponding with the duration of gestational periode in rat is 21-23 days from copulation to parturition.¹⁸ Measurement for maternal body weight and blood pressure at 21th day of treatment and then collected of blood for measurement: MDA, glucose, creatinine and erythrocyte level before sectio caesarean procedure for calculated number of offspring. MDA is measured by TBARS (*Thiobarbituric Acid Reactive Substances*) using wave length 230 nm Spectrophotometry.

RESULT

Iron-folate supplementation were given during pregnancy had better results than the control group that did not received supplementation. Increasing dose of iron folate supplementation better than the lowest. Level of oxidative stress level were lowest at group (III) who received one half recommended doses was marked by the lowest MDA levels. Low levels of oxidative stress are associated with optimizing organ function in pregnancy characterized by better excretion system, energy synthesis and hematopoietic system. The highest level of oxidative stress in control group. Creatinine levels were lowest in group III, followed by group II, group I and highest in the control group. The highest number of erythrocytes in group III and the lowest in the control group, the number of children produced and the highest maternal weight in group III and the lowest in the control group. The highest glucose level was in the control group while the lowest was in group III (Table 1)

Table 1: The effect of different concentration iron and folate supplementation on pregnancy outcome.

Sample	Control (C)	K I	K II	K III	P
Number of offspring ¹	5,8±0,89	7,0±0,71	10,0±0,71	10,8±0,84	0,001
Birth weight (g) ¹	3,69±0,38	4,85±0,40	5,65±0,26	6,52±0,37	0,001
MDA level (µmol/L) ²	5,94 ±0,71	3,5±0,64	2,93±0,36	2,34±0,21	0,001
Creatinine level (mg/dL) ²	3,18±0,12	0,99±0,04	0,77±0,04	0,64±0,46	0,001
maternal boddy weight (g) ²	219,2±5,63	239,0±3,53	245,0±4,18	247,4±4,22	0,001
Glucose (mg/dL) ²	134,6±3,42	97,4±2,49	89,6±1,93	82,2±3,18	0,001
erythrocyte (x10 ⁶ /µL) ²	6,6±0,12	8,9±0,04	8,34±0,23	10,3±0,45	0,001
blood pressure (mmHg) ²	138,6±4,51	105,6±4,56	94,4±2,70	88,4±5,41	0,001

1. Offspring
2. Maternal

DISCUSSION

The results of this study indicate that iron and folate deficiency during pregnancy increases oxidative stress and iron folate supplementation has a significant effect to reduce oxidative stress marked a significant decreases in MDA levels between the control group that did not receive iron folate supplementation compare to the treatment group that received supplementation with half doses, recommended doses and one half of the recommended doses of iron folate supplementation during pregnancy.

The treatment group that received iron and folate supplementation resulted MDA and glucose levels were lower compared than controls, and an increase in iron and folate doses resulted in lower MDA and glucose levels. Iron and folate supplementation has been shown to reduce level of oxidative stress and this impact to reduce glucose levels. Low level of ROS are involved in cell signalling pathways, important to cellular function but ROS that presence of in higher level can lead to cellular damage of DNA, lipids and cellular proteins. Increased ROS would be increased MDA level³

This study was found that glucose levels and MDA level in the control group higher than the treatment group. These results are in accordance with the statement of Keane *et al.*, 2015 that the level of oxidative stress and inflammatory processes are the main key role of physiological and pathophysiological processes related to insulin resistance and the progression of DM type2. Increased free radicals can trigger inflammatory reaction followed by migration and infiltration of macrophages in peripheral tissues including the pancreas, liver and adipose tissue. Impaired tissue function can reduce insulin secretion and then glucose level were increased. TNF- which is an inflammatory mediator suppresses the activity of insulin receptors through serine phosphorylation in IRS1 (insulin receptor substrate-1), which is an important signaling protein for decreasing insulin receptors. One of the first consequences of inflammation is insulin resistance, since TNF- α prevents the phosphorylation of insulin receptors, interfering in their cascade action and preventing their functioning.^{19,20}

Iron folic supplementation during pregnancy has been shown to improved the outcome of the pregnancy process characterized by lower level of creatinine, glucose and blood pressure at the supplementation group compared to the control group. There were improving metabolism in the body marked by significant differences in maternal body weight at the end of pregnancy. The improvement of metabolic processes in the body was also evidenced by the significantly higher number of offspring and birth weight of offspring between the control group and iron-folate supplementation group.

The results of this study are supported by research conducted by Li, *et al.* 2019 who found that intra-uterine hypoxia can affect pregnancy outcomes and then could be inhibit intra-uterine growth, cardiovascular system dysfunction and multi-organ abnormalities such as the brain, heart, liver, and kidneys.⁸ The mechanism underlying multiorgan abnormalities in the fetus is hypoxia intra uterine affecting fetal programming, lack of oxygen can spur mitochondria to produce superoxide ions and increase oxidative stress. Oxidant molecules can interact directly with DNA bases causing genetic and epigenetic abnormalities through DNA methylation, histone modification, chromatin remodeling, and noncoding RNA. Changes in oxidation-reduction status (redox status) have an effect on gene expression and methylation of DNA that develop into suppression of the transcription process of mRNA and protein synthesis. ROS can directly interact with DNA causing damage to DNA. Damage to oxidant-mediated DNA can also attach to the DNA methyltransferase side and then trigger the DNA methylation process. DNA oxidative damaged might be block the binding of DNA methyltransferase (DNMT) to the DNA template; activation of transcription factors (TFs) can inhibit DNMTs

from accessing the DNA, resulting in gene-specific hypomethylation; interfering activity of DNMT or/and ten-eleven translocation (TET) enzyme families, leading to genomic methylation dysregulation.^{5,8} DNA methylation can play a key role in local control of gene expression.⁸ This study also supported by research conducted by Liu, *et al.* 2018 that found supplementation of multivitamins containing folic acid during pregnancy could significantly lower preeclampsia risk.¹⁵

Modification of gene expression can also occur due to changes in regulatory function in histones, especially in the terminal amino groups that are susceptible to changes in posttranslation modification such as methylation, acetylation, phosphorylation and ubiquitination. Changes in the methylation pathway that cause a decrease in protein synthesis and methylation of DNA result in abnormalities of vascular smooth muscle proliferation and increase lipid peroxidation.²¹

Folic acid deficiency causes the body to be unable to produce methionine resulting in various problems such as low production of natural antioxidants (glutathione) and amino acids containing sulfur (taurine and cysteine) which play a role in the process of eliminating toxins from the body, strength and health of the tissue, improving the health of the cardiovascular system, low levels of methionine cause liver function failure characterized by fatty acid deposits, failure of creatine production in muscles, methionine is also an important component in the formation of collagen, skin, nails and connective tissue.¹⁴ Folic acid has been associated with global measures of methylation in infants, children, and adults. This effect appears to be limited to the third trimester of pregnancy. Meanwhile, increased maternal intake of methyl donor nutrients has been associated with increased global methylation in the cord blood of offspring, whereas increases in maternal methyl donors influence methylation patterns related to infant metabolism and growth.¹⁶ Genome-wide studies have shown altered DNA methylation in tissues important for glucose homeostasis including pancreas, liver, skeletal muscle, and adipose tissue from subjects with type 2 diabetes compared with nondiabetic controls.⁷

Folic acid works together with vitamin B12 in the formation of blood cells and helps function iron in the body. Folic acid, vitamin B6, and vitamin B12 and other nutrients function to control homocysteine levels in the body. Folic acid has a vital role in the mechanism of action in the human body. Growth and cell development through various reactions which include histidine cycles, serine and glycine cycles, methionine cycles, thymidylate cycles, and purine cycles and participate in the process of transporting K⁺ ions into spinal fluid and across the brain barrier. The condition of folic acid deficiency causes all cycles to become inactive and can cause problems such as megaloblastic anemia, cancer, and neural tube defects. Folic acid deficiency causes disruption due to fat, carbohydrate and protein metabolism, impaired DNA and RNA synthesis, impaired brain and central nervous system function, psychological disorders such as depression, anxiety, insomnia and immune system disorders.¹⁴

Creatinine serum levels in the group that treated with iron folate supplementation were lower than the control group showed the role of folic acid in optimizing kidney function. These results are consistent with the study,¹² in Hhcy mice induced by a folate-free diet provides glomerular sclerosis characterized by local oxidative stress, causing apoptosis of mesangial cells through the production of mitogen ROS and p38 activated by protein kinase, mesangial expansion, podocyte dysfunction and fibrosis. This disorder improves with the administration of apocynin which is an NADPH oxidase inhibitor.^{11,12} Increased levels of Hcy associated with inflammation of the kidneys are shown by the expression of MCP-1 in the glomerular monocyte chemoattractant. Hcy (50–200 μ M) induces increased levels of MCP-1 protein and mRNA in glomerular MC through NF- κ B activation, this process results in increased production of stress oxidative.¹¹ Research carried out on HHcy mice MCP-1 protein, and increased mRNA levels

in the kidneys and this increase depends on NF- κ B. The results of this study support the concept of HHcy increasing the progression of kidney disease caused by inflammation. Yi, *et al.* showed a significant increase in the expression of NOD2 in HHcy rat kidneys accompanied by an increase in proinflammatory cytokines consisting of: IL-1 β , IL-6, TNF- α , MCP-1, and intracellular adhesion molecule-1 (ICAM-1). Hcy causes endoplasmic reticulum stress in proximal tubular cells by severing disulfide bonds that damage protein bonds, Hcy activates gene expression: BiP / GRP78 which functions to control signaling pathways against load responses in endoplasmic reticulum, CHOP/GADD153 which encodes transcription factors that influence the Endoplasmic Reticulum stress response. Another mechanism for Hcy to induce cell damage through Endoplasmic Reticulum is probably related to activation of IRE-1, signaling molecules that accelerate JNK protein kinase activity and cell apoptosis.¹¹ Hhcy is known to play a role in the progression of vascular damage supported by an increase in the inflammatory process and endothelial injury that develops into cardiovascular disease and chronic kidney disease. Folic acid is also useful in improving endothelial function without reducing Hcy.¹³ Mechanisms underlying the role of HHcy in the pathogenesis of disease through oxidative stress, inflammation, Endoplasmic Reticulum Stress, and DNA hypomethylation. Other mechanisms that might play a role in the pathogenesis of diseases associated with HHcy are elevated levels of Hcy thiolactone, homocysteinylated, mitochondrial dysfunction.¹¹

Average of maternal body weight, number and body weight of offspring at control group significant lower than treated group with iron-folate supplementation, this condition shows that the role of iron and folic acid in the growth and development of the fetus intra uterine. This result is in accordance with epigenetic dysregulation triggered by environmental cues during these sensitive periods of individual development can persist across the life course leading to altered disease susceptibility and even phenotypic changes.⁸ The results of this study support the study of Ahmed *et al.*, 2011 folate deficiency in serum is related to birth weight (OR 11.0, 95% CI 4.81-25.15), lower length (OR 3.67, 95% CI 1.42-9.47).²² This study support study of Balarajan *et al.*, 2013. They found that IFA during pregnancy was negatively associated with LBW after adjustment for socioeconomic status [OR = 0.77 (95% CI: 0.68, 0.87) ; P < 0.001] and further adjustment for antenatal care (ANC) utilization [OR = 0.82 (95% CI: 0.72, 0.94); P < 0.001]. This corresponded to a 41-g [95% CI: 2 g, 80 g]; P < 0.05]. IFA during pregnancy was significantly associated with decreased LBW.²³

Glucose levels in the group that were treated with iron-folate supplementation were lower compared to control group. this results according to the Pravenec studies in SHR (spontaneously hypertensive rat) which showed that folate deficiency can promote oxidative stress and multiple features of the metabolic syndrome that are associated with increased risk for diabetes and cardiovascular disease. Reduced folate levels were correlated with approximately 3-fold greater levels of total plasma homocysteine whereas cysteine concentrations were lower in with folate deficiency.²⁴ TNF- α which is an inflammatory mediator suppresses the activity of insulin receptors through serine phosphorylation in IRS1 (insulin receptor substrate-1), which is an important signaling protein for decreasing insulin receptors and then increase glucosa level.¹⁹

Rats fed a folate-deficient diet showed decreased levels of glutathione in all 3 tissues included : liver, heart, and kidney that showed evidence of oxidative tissue damage as reflected by increased concentrations of lipoperoxidation products including conjugated dienes and/or thiobarbituric acid reactive substances. Activity of the antioxidant enzymes CAT, SOD, and GSH-Px also decreased in folate deficiency diet and then increased lipid peroxidation which is characterized by increased levels of MDA.^{19,24} This result of studies also support the Guest research that found in the peripheral circulation there were observed

positive associations between plasma folate and plasma TAC (*Total Antioxidant Capacity*) levels ($P \leq 0.01$) and plasma NAD(H) ($P \leq 0.05$). Plasma folate was inversely associated with plasma Hcy concentrations ($P \leq 0.05$).²⁵ Iron is an important part of the antioxidant system. Levels of anti-oxidant enzymes were significantly lower ($p < 0,001$), while lipid peroxidation were increased in children with IDA (*Iron Deficiency Anaemia*) compare to controls²⁶

Both iron deficiency and iron overload can affect redox state, and these conditions can be restored to physiological conditions using iron supplementation and iron chelation, respectively iron metabolism underlies the dynamic interplay between oxidative stress and antioxidants capacity in many pathophysiological processes. The conditions that give rise to iron deficiency also promote oxidative stress, the hypoxic condition induced by anaemia may worsen oxidative stress via pro-oxidant changes.²⁷

CONCLUSION

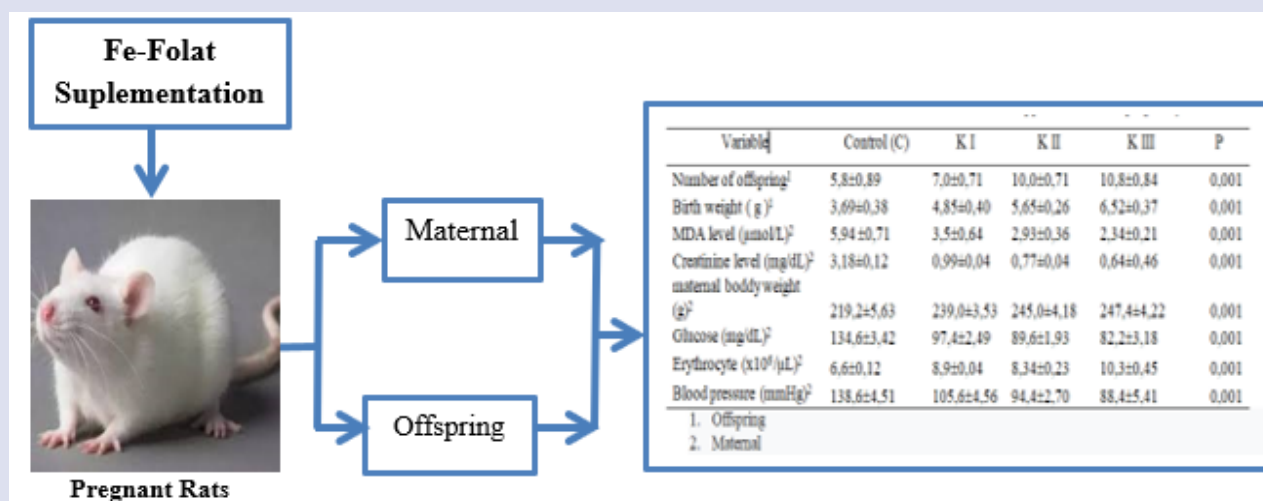
Iron-folate supplementation during pregnancy can affect the health status of both the maternal and the offspring. Iron folate supplementation can improve the antioxidant system and consequently improve energy metabolism, hematopoietic function, kidney function, etc in the body characterized by an increase in maternal body weight during pregnancy, an increased in the average number of offspring and fetal body weight, an increase in erythrocyte levels, a decreased in MDA, creatinine and glucose levels. Iron and folate supplementation during pregnancy can decreased level of oxidative stress and this consequence of better pregnant outcome.

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GRAPHICAL ABSTRACT



SUMMARY

Effect of iron-folate supplementation during pregnancy for maternal were reduced level of: MDA, creatinene, glucose, blood pressure and increased level of erythrocyte and body weight. Effect of iron-folate supplementation during pregnancy for offspring were increased both average number and fetal body weight.

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Cite this article: Wahyuwibowo J, Aziz A, Safitri E, Minidian F, Zulaikhah ST. Iron-Folate Supplementation during Pregnancy for Prevent Oxidative Stress in Pregnant Rats: Level of MDA, Creatinine, Glucose, Erythrocyte, Blood Pressure, Body Weight and Number of Offspring. *Pharmacog J.* 2020;12(1):186-91.