

## Effect of aqueous moringa seed extract on oxidative stress in Alloxan- induced gestational diabetic rats

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### ABSTRACT

The mechanistic pathway for the antioxidant efficacy of the aqueous *Moringa oleifera* seed extract in gestational diabetes was investigated by evaluating five antioxidant parameters, three (Malondialdehyde, Glutathione-S-Hydrogenase and Glutathione-S-Transferase) of which were done from the uterus and two (Catalase and Super-oxide-dismutase) from the serum using a pregnant diabetic animal model. Pregnant Wistar rats were divided into four groups of 8 each made up of Normal control untreated, Non-diabetic treated, Diabetic treated and Diabetic untreated. The four groups of animals were used to evaluate the effect of the extract on the biochemical parameters and the level of antioxidants in the uterus and the serum. The level of significance of the level of biochemical parameters were determined respectively using the Analysis of variance.

The administration of 300mg/kg body weight of *Moringa oleifera* seed extract produced significant increase in Catalase and Super-oxide-dismutase levels in the serum. However, there was a significant decrease in the uterus of Malondialdehyde and a significant increase in the uterus of Glutathione-S-Hydrogenase and Glutathione-S-Transferase when compared with the normal untreated group and the untreated diabetic groups.

These observations support the hypothesis that aqueous *Moringa oleifera* seed extract possesses antioxidant parameters that are able to counter or revert the effect of gestational diabetes induced by oxidative stress.

Key Words: Gestational diabetes, Moringa, Antioxidants, Oxidative Stress, Alloxan

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### **INTRODUCTION**

The role of oxygen in metabolism has made it one of the elements necessary for the maintenance of life. Yet oxygen is a highly reactive molecule that damages living organisms through the production of reactive oxygen species (ROS) (1). Organisms however contain complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components like DNA, proteins and lipids (2,3). Oxidative stress has been known to contribute to the development of a wide range of diseases including Alzheimer's disease (4) and many pregnancy related disorders (5). ROS and antioxidants have been implicated in the regulation of reproductive process in both human and animals (6). Imbalances between ROS production and antioxidants systems produce oxidative stress that negatively impacts reproductive process thus promoting many pregnancy related disorders. (6,7). During pregnancy, metabolic adaptations are necessary to ensure the growth and developments of the fetus and to meet the altered demands of the mother. Glucose is the major substrate for the human fetus throughout pregnancy. Insulin sensitivity decreases while its secretion increases. This process stabilizes the glucose input to the fetus. Decreased maternal insulin sensitivity during pregnancy can however become a health liability when food nutrient is in abundant supply accompanied by a sedentary lifestyle thus producing hyperglycemia with a long-time risk of obesity and diabetes in both mother and fetus. The pathogenesis of diabetes during pregnancy and the possibility of its management with therapeutic agents without side effect has been the subject of past studies. (8) but yet the management of gestational diabetes is still a challenge to the medical system. The World Health Organization recommended and encouraged

the use of alternatives especially in countries where access to conventional treatment is not adequate (9). Herbs such as bitter lemon, garlic, okra and psyllium seeds have been known to possess antidiabetic activities when administered concurrently with allopathic drugs (10).

*Moringa oleifera* belongs to the family Moringaceae. The plant is widely distributed in many tropical and subtropical countries. Different parts of the plant including the seeds have found alternative means of treatment of various diseases including diabetes. (11) The paucity of scientific data on the effect of *Moringa oleifera* on induced gestational oxidative stress, despite its vast therapeutic influence and application prompted this study.

The aim therefore of this study is to establish the efficacy of *Moringa oleifera* seed extract in the treatment of alloxan-induced gestational diabetes mellitus and to evaluate the potency of the antioxidant parameters in the treatment of alloxan-induced gestational oxidative stress.

### **2.0 MATERIAL AND METHODS**

#### **2.1 Experimental Animals**

Fifty Wistar rats with average weight of 150 gm were used in the experiment. The animals were all housed under the same experimental and environmental condition with the provision of 12 hours of light and darkness. The rats were fed with growers mash containing 16.50% of crude protein, 4.3% of crude fiber and 4.4% of crude fat. Water was supplied ad libitum. The weights of the animals were monitored throughout the period of the experimentation.

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### **2.2. Aqueous Extraction of Moringa seed.**

Meanwhile, *Moringa oleifera* seeds were obtained from a known farm in Ogbomoso, Oyo State, Nigeria. The matured seeds were separated manually from the husks. The seeds were air-dried for three weeks and pulverized to fine powder by a mixer grinder. The aqueous moringa extract was produced from 450gm of the powder using the Soxhlet extraction method ((J. SIL Borosilicate). Briefly described, 400mls of normal hexane was poured into a round bottom flask. Twenty grams of the *Moringa oleifera* powder was placed in the thimble of the Soxhlet extractor on top of the flask which was heated at 60<sup>0C</sup>. The distillate was collected in the flask, dried in the oven, cooled in the desiccators and weighed to determine the amount of extract. This procedure was repeated until all the 450 gm of the *Moringa* powder were distilled. At the end of the extraction, the resulting mixture was distilled off using simple distillation to recover the extract from the solvent. The extracts were stored at 4<sup>0C</sup> until ready for use.

### **2.3 Grouping of the animals**

The fertility period of all the animals were determined by the vaginal smear method and were all observed to be on heat. The animals were thereafter divided into four groups of 15 animals each and housed in wire meshed cages. Three male rats were introduced into each of the cages for copulation but were withdrawn after the third day. Pregnancy was determined by the presence of copulatory plug and observation of the enlargement of the lower abdomen. Rats that were not pregnant were later excluded from the experiment while the pregnant rats were eventually divided into four groups of 8 rats each.

Group 1 animals served as the control group while Groups 2-4 animals served as the experimental groups. Group 1 animals were

given distilled water only. Group 2 animals received orally through a cannula 300mg/kg body weight *Moringa oleifera* extract. Group 3 animals contained animals with induced diabetes. Diabetes was induced by intraperitoneal injection of 150mg/kg body weight of alloxan and fed orally with *Moringa oleifera* extract at 300mg/kg body weight. Group 4 animals were treated the same as Group 3 except that the animals did not receive the extract.

The 18 hour fasting glucose test was performed on all the groups using the ACCU-Chek glucometer and test strip (Johnson-Johnson, California, USA) to establish the normal glucose level before gestation. Blood samples were obtained from the caudal vein of the conscious rats and dropped on the active ACCU-Chek strips. The basal and the 48-hour post diabetic induction blood glucose levels were determined in mg/dl. Weekly blood glucose levels were also recorded during treatment with extract till the animals were sacrificed.

On each occasion when diabetes was induced, the induction was done on the seventh day after gestation while blood glucose was determined on the tenth day after gestation. Administration of *Moringa oleifera* extract commenced on the eleventh day and lasted till term.

### **2.4 Blood and uterus collection.**

Three weeks after oral administration of extracts, the rats were sacrificed and the uterus were harvested while the blood was collected by cardiac puncture. The uterus was weighed and homogenized in phosphate buffered saline (PBS) 1:5 ratio (w/v). Plasma was separated from the blood by low grade centrifugation at 250-300 rpm. Sera were stored in 0.1ml volumes and kept at 4<sup>0C</sup>

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alongside the uterus homogenate till ready for use.

### **2.5 Determination of antioxidant parameters in the serum.**

Catalase (CAT) activity of the serum was measured by the method of Aebi,(12). Briefly described, a 1:20 suspension (v/v) of the serum was made in 50mM PBS, pH 7.0 and 1 ml of freshly prepared 30% H<sub>2</sub>O<sub>2</sub> was added to initiate the reaction. The rate of decomposition of the H<sub>2</sub>O<sub>2</sub> was measured in the spectrophotometer at an absorbance of 240nm.

Super-oxide-dismutase (SOD) was determined by the method of Misra and Fridovich (13). About 2.8ml of the cocktail containing 3.4x10<sup>-6</sup> M epinephrine; 0.05M Na<sub>2</sub>CO<sub>3</sub>, pH10.2 and 1x 10<sup>-4</sup> EDTA was pipetted into the cuvette and about 0.2 ml of the serum was added. Absorbance was recorded at 480 nm. Enzyme activity was expressed in nm.

### **2.6 Determination of antioxidant parameters in the uterus.**

Malondialdehyde(MDA) was determined based on the principle of Varshney and Kale (14). Estimation of lipid peroxidation is based on the reaction of MDA with barbituric acid (TBA) combining to form an MBDA-TBA adduct that absorbs strongly at 532nm. About 0.4 ml of a reaction mixture containing the uterus homogenate, quenched with 30% Trichloroacetic acid(TCA), was added to 1.6ml of Tris KCl and TBA was then added. The mixture was centrifuged at 1400g for 15 minutes. Absorbance was measured at 532nm.

Glutathione -S-hydrogenase was determined based on the principle of Jollow et al.(15). Ten percent of uterus homogenate was mixed with sulphosalicylic acid in a 1:1 ratio and incubated at 40<sup>OC</sup> for 1 hour. Absorbance of the mixture was read at 412 nm

Glutathione -S- Transferase activity was done according to the method of Habig (16). 0.1ml of 30mM was pipetted into cuvette containing 0.1 ml of 1-chloro-2,4 dinitrobenzene and PBS, pH 5.6. Reaction was initiated by the addition of 0.6 mls supernatant uterus homogenate. Absorbance was measured at 340nm.

### **2.7. Statistical Analysis**

Statistical analysis was by the two-factor with replication analysis of variance (ANOVA) and by the t-test paired two samples for means. Results were expressed as means ±SD. P≤ 0.05 was considered statistically significant.

## **3.0 RESULTS**

Thirty two of the forty rats (80%) were confirmed pregnant and were divided in four cages of eight each. The pre-induction glucose level in all the four groups was between 71mg/dl and 109mg/dl while the post -induction glucose level was between 93mg/dl and 139mg/dl. There was an observed difference in the increase of glucose level between the pre-induction and post induction animals. All the animals experienced increase in glucose after induction but it is only in Group 3 animals that pre- and post-induction glucose level was significant (p<0.05). (Tables 1 and 2)

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<b>Table 1. Glucose Level of Animals Before and After Diabetes Induction</b>					
<b>T-Test: Paired Two Sample for Means</b>					
	Pre-Induction (N=8)		Post-Induction (N=8)		Sig.
	Mean	(Standard Deviation)	Mean	(Standard Deviation)	
Control (Group 1)	86.88	(4.97)	116.88	(4.97)	p>0.05
Not Diabetes Induced but Treated (Group 2)	92.25	(11.15)	122.25	(11.15)	p>0.05
<b>Diabetes Induced and Treated (Group 3)</b>	<b>84.63</b>	<b>(9.68)</b>	<b>115</b>	<b>(10.21)</b>	<b>P(0.00000182)&lt;0.05</b>
Diabetes Induced but not treated (Group 4)	83.88	(16.34)	113.88	(16.34)	p>0.05

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<b>Table 2. Glucose Level of Animals Before and After Diabetes Induction</b>										
<b>ANOVA: Two-Factor With Replication</b>										
	Pre-Induction (N=8)		Post-Induction (N=8)		Group 1 vs Group 2 vs Group 3 vs Group 4		Pre-Induction Vs Post-Induction		Interaction [Time (pre-and post-induction) by Group]	
	Mean	(Standard Deviation)	Mean	(Standard Deviation)	F-Value	Sig.	F-Value	Sig.	F-Value	Sig.
Control (Group 1)	86.88	(4.97)	116.88	(4.97)	1.75	p>0.05	112.57	P(0.000000000000000512)<0.05	0.001	p>0.05
Non Diabetes Induced but Treated (Group 2)	92.25	(11.15)	122.25	(11.15)						
<b>Diabetes Induced and Treated (Group 3)</b>	<b>84.63</b>	<b>(9.68)</b>	<b>115</b>	<b>(10.21)</b>						
Diabetes Induced but not treated (Group 4)	83.88	(16.34)	113.88	(16.34)						

Results of the antioxidant parameter tests are presented in Table 3 and Figures 1 and 2.

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### **3.1 Malondialdehyde (MDA).**

MDA level in the uterus was 0.022  $\mu\text{m}$  in the normal control untreated group 1 and 0.036  $\mu\text{m}$  in the non-diabetic treated group 2 and 0.46  $\mu\text{m}$  in the diabetic untreated group 4 animals. The value in group 3 diabetic treated animals was 0.023  $\mu\text{m}$ , very close to the normal control group. (Table 3, Fig 1.). Values were significant ( $p < 0.05$ ) when compared with the antioxidant level of the control group. However, there was no significant difference in the MDA level across the group ( $p < 0.05$ ) as there was a significant increase in the diabetes untreated group 4 when compared with diabetes treated group 3 ( $p < 0.05$ ).

### **3.2 Glutathione-S-Hydrogenase (GSH).**

The GSH antioxidant level as obtained in uterus was 0.33  $\mu\text{m}$  in the normal control group 1, 0.42  $\mu\text{m}$  in the non-diabetic treated group 2, 0.37  $\mu\text{m}$ , in diabetic treated group 3 and 0.35  $\mu\text{m}$ , in the diabetic non-treated group 4 (Table 3, Fig.1). Values were significant ( $p < 0.05$ ) when compared with the antioxidant value of the control untreated group. There was no significant difference across the group ( $p < 0.05$ ) as there was a significant increase in the level of GSH in the non-diabetic treated animals of group 2 when compared with the untreated group ( $p < 0.05$ ).

### **3.3. Glutathione-S-Transferase (GST).**

The mean GST level in the uterus ranged from 25.31  $\mu\text{m}$ , in the diabetic untreated group 4 and 31.67  $\mu\text{m}$  in the non-diabetic treated group 2 (Table 1, Fig 1). Although

there were differences in the values, there was however no significant difference ( $p < 0.05$ ) when compared with the antioxidant of the normal untreated control group 1. Levels were also not significant when the groups were compared with one another. Level of antioxidants in the serum are also presented.

### **3.4. Catalase (CAT).**

The antioxidant Catalase level was lowest in the diabetic untreated group 4 with the value of  $62.63 \pm 5.423 \mu\text{m}$ , and 78.0  $\mu\text{m}$ , 78.3  $\mu\text{m}$ , and 77.8  $\mu\text{m}$ , in the normal control untreated, non-diabetic treated group 2 and the diabetic treated group 3 respectively. (Table 3 Fig 2.). When all the groups were compared with one another, the values were insignificant, but there was a significant difference when the value of the diabetic untreated group 4 was compared with both the control and the treated groups ( $p < 0.05$ ).

### **3.5 Super oxide dismutase (SOD)**

This antioxidant was lowest in the diabetic untreated group 4 with a value of 11.9  $\mu\text{m}$ . The diabetic treated group had an SOD level of 34.5  $\mu\text{m}$ , while the normal control untreated group 1 and the non-diabetic treated group 2 had values of 36.0  $\mu\text{m}$ , and 58.0  $\mu\text{m}$ , respectively. These values were significant across the group as there was a significant decrease of SOD level in the diabetic untreated level 4 when compared to the normal control untreated. ( $p < 0.05$ ). Also, there was a significant increase in the SOD level of the moringa treated diabetic group 3 when compared with the diabetic untreated group 4. ( $p < 0.05$ ). (Table 3, Fig.2)

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**Table 3. Level of Four Antioxidants in the Uterus and Sera of Pregnant Diabetic and Non-Diabetic Wistar Rats.**

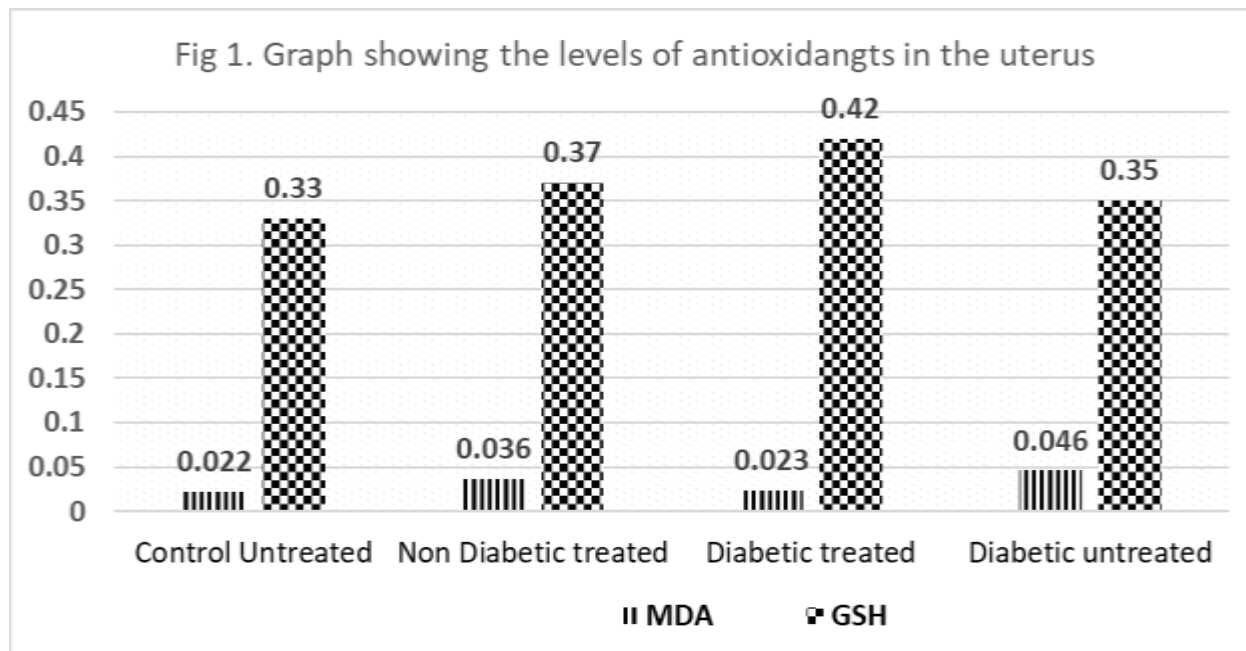
Antioxidants	Antioxidant Levels in control and experimental Rats ( $\mu\text{m}$ )			
	Normal Control	Non- Diabetic Treated	Diabetic Treated	Diabetic Non- Treated
	1	2	3	4
Malondialdehyde(MDA)	0.022 $\pm$ 0.004	0.036 $\pm$ 0.014	0.023 $\pm$ 0.006	0.046 $\pm$ 0.003*
Glutathione -S-hydrogenase (GSH)	0.33 $\pm$ 0.02	0.42 $\pm$ 0.04*	0.37 $\pm$ 0.03	0.35 $\pm$ 0.02
Glutathione-S-Transferrase (GST)	26.35 $\pm$ 3.73	31.67 $\pm$ 7.34	28.54 $\pm$ 8.85	25.31 $\pm$ 2.43
Catalase(CAT)	79.19 $\pm$ 4.064	80.65 $\pm$ 3.972	76.52 $\pm$ 4.106	62.63 $\pm$ 5.423*
Superoxide dismutase(SOD)	39.18 $\pm$ 3.232	59.70 $\pm$ 9.391*	38.25 $\pm$ 5.571	13.06 $\pm$ 6.188

Values are expressed as means  $\pm$ SD (n=8 number of rats)

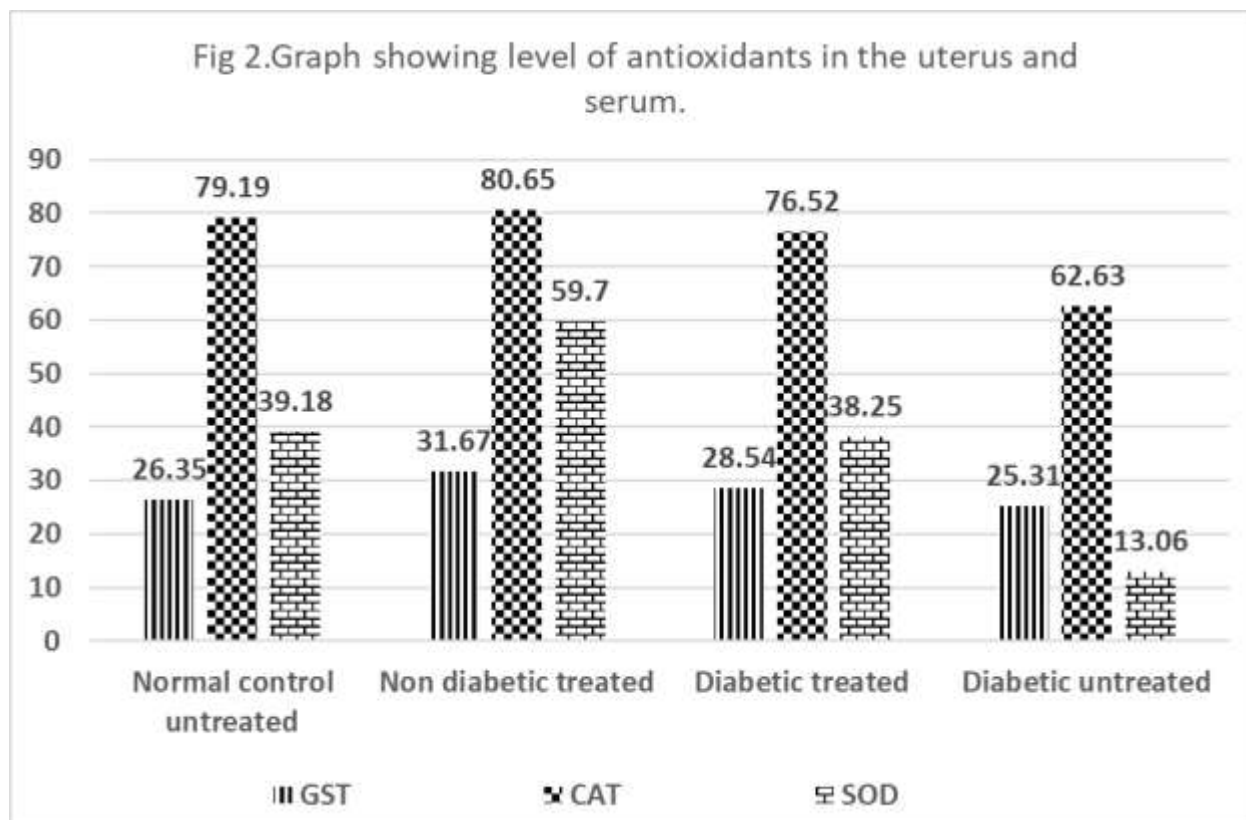
\*Values were significant (p<0.05) when compared with the antioxidants of the control group.



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### **Discussion and Conclusion.**

This study has attempted to determine the antioxidant effect of *Moringa oleifera* seed extract on pregnant Wistar rats induced with alloxan to produce gestational diabetes mellitus by generating oxidative stress radicals. Oxidative stress damages the  $\beta$ - cells of the Islets of Langerhans of the pancreases (17), predisposing to a state of hyperglycemia. Antioxidants can terminate these chain reactions by removing the free radical intermediates (18) and so in this study we have used five of the many antioxidants known to be present in the plant *Moringa oleifera* (19) to investigate the dynamics and treatment of gestational diabetes mellitus in Wistar rats. Results obtained showed that there was no significant difference in both the level of SOD and CAT of the gestational groups that were treated with the extracts when compared with the normal control untreated animals. There was however a significant difference ( $p < 0.05$ ) when the same groups were compared with the untreated gestational diabetic groups. Administration of *Moringa oleifera* seed extract to the pregnant rats significantly increased the level of both these antioxidants ie SOD and CAT in the serum of these rats that received the extract. These findings agree with the findings of Siddhuraju and Becker (20) who observed that *Moringa oleifera* seed extracts were capable of scavenging peroxy and superoxy radicals by increasing the catalase and SOD activities. Results also showed a significant increase in the level of MDA in the uterus of the untreated diabetic groups. Gomez-Zubeld et al. (21) in a similar study observed the same finding and suggested that uterine MDA levels tend to rise above normal in oxidative stress. In this study, we observed that *Moringa oleifera* seed extract revert the effect of oxidative stress in the uterus by decreasing the MDA level in the uterus. During pregnancy, the synthesis rate of lipoperoxides

exceeds their decomposition rate, which often leads to oxidative stress. Lipoperoxides also increase in the fetus as it develops (22). It is therefore obvious that the decrease in the level of the antioxidant MDA in the uterus serves as proper antioxidant defense of the fetus. (23). This hypothesis is supported by the result obtained in this study.

GSH and GST increased in the uterus of the treated diabetic group and the treated non-diabetic group compared to the control and non-treated group. This suggests that modulation of induced diabetic oxidative stress with aqueous *Moringa oleifera* seed reduces uterine damage at the biochemical level. This observation is in agreement with the findings of Guney et al. (24) and Sunghal et al. (25). Women with gestational diabetes are at high risk of maternal complications during pregnancy. Impaired placental function has been found to contribute to GDM during pregnancy. Placenta hinders insulin signaling and this adversely affects the placental transport of insulin and metabolism of glucose as well as lipid, all these giving rise to negative impacts on fetal growth and development (26). Studies on experimental animals have shown the important role of oxidative stress and lipid peroxidation in the development of fetal malformation associated with gestational diabetes (27,28).

The pathogenesis and spectrum of fetal and neonatal mortality and morbidity have been found to be primarily attributed to the excessive transfer of glucose from mother to fetus (29). In this study, a onetime induction of the animals with 150mg/kg body of alloxan showed a difference in the level of post induction glucose level between the groups and the pre-induction glucose level. Although the post induction glucose levels of the four groups of the animals were higher than their respective pre-induction glucose level, this difference was more significantly pronounced in the pre-induction and post induction

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glucose level of the animals in treated diabetic induced group 3 animals. This suggests that *Moringa oleifera* seed extract is capable of reducing the level of glucose intolerance induced by alloxan in rats and the subsequent oxidative stress effect. The significant reduction of the glucose level in the diabetic treated group in this study suggests that application of *Moringa oleifera* extracts may play a role in the reduction of morbidity and mortality of fetus and neonates and in the amelioration of the general pathogenesis of GDM. This area calls for further attention in any future studies or clinical trials. Since this study was limited to the evaluation of the efficacy and potency of antioxidant parameters of *Moringa oleifera* extracts in the treatment of alloxan induced gestational diabetes, no attempt was made to observe any pathological changes in the uterus or fetuses. However, infants of mothers suffering from GDM have been found to be vulnerable to major health problems during pregnancy including macrosomia, hypoglycemia, respiratory distress and oxidative stress. (26). It will be interesting to follow up this study with such investigation to determine the comparative reduction in such occurrences following the administration of *Moringa oleifera* extracts and therefore further studies are suggested in this area. Likewise, only five of the known antioxidants present in *Moringa oleifera* were selected for investigation in this study. For a more conclusive result and observation, it is suggested that further

investigations be conducted with more of the known antioxidants to determine any synergism or antagonism in the treatment of gestational diabetes mellitus. Strain (30) observed in an earlier study some disturbances between micronutrients and antioxidants in some diabetic cases.

### **Conclusion.**

This study has demonstrated that the administration of aqueous *Moringa oleifera* seed extract to an oxidative stress induced diabetic rats led to significant increase in the serum CAT and SOD levels and an increased GHS and GST levels in the uterus and subsequent decrease in the MDA level in the uterus suggesting that *Moringa oleifera* contains antioxidants that are potent and capable of reverting gestational diabetes. If the same result is reproducible in humans, there is no doubt that *Moringa oleifera* seed extracts will find important application in the alternative medicine treatment of gestational diabetes in humans.

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### **Conflict of Interest.**

There is no conflict of interest.

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### References

1. Davies, KJ. Oxidative stress: The paradox of aerobic life. *Biochemical Society symposia* 61: 1995; 1-31.
2. Sies, H. Oxidative stress: Oxidants and antioxidants. *Experimental Physiology* 1997; 82(2): 291-295.
3. Vertuani, S; Angusti, A; Manfredini, S. The antioxidants and Pro-Antioxidants Network: An overview. *Current Pharmaceutical Design*. 2004, vol 10(14): 1677-1694.
4. Christen, Y. Oxidative stress and Alzheimer disease. *Amer Jour Clin Nutr*. 2000;71(2) ;621S-629S
5. Davi, G, Falco A., Patrono C. Lipid peroxidation in diabetes mellitus. *Anatioxid Redox Signal*. 2005; 71(1-2) :2
6. Al-Gubory, KH, Fowler PA, Garrel C. The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. *Int J Biochem Cell Biol*. 2010 Oct; 42(10):1634-50.
7. Agarwal A<sup>1</sup>, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. *Reprod Biol Endocrinol*, 2005 Jul 14;3:28
8. Kristina, B., Nanmi S., Kota, MK, and Kristina Rao RV. Evaluation of antihyperglycemic effects of Datura metal lim seeds in normal and alloxan induced rats. *Journal of Ethnopharmacol*. 2004; 9:95-98.
9. World Health Organization. Dept of non-communicable disease surveillance. Definition and classification of diabetes mellitus and its complications. Reports. 1999. WHO. Geneva.pp. 1-33.
10. DeSmet, PAGM and D'Arcy, PF. Drug interaction with herbal and non orthodox remedies. In: D'Arcy PF, McElna JC, Welling PG, eds *Mechanism of drug interactions* New York NY: Springer-Verlag:327-352.
11. Kar, A.Chouldhary BK, Bandyopadhyay NG. Comparative evaluation of hypoglycemic activity of some indian medicinal plants in alloxan diabetic rats. *Jour. Ethnopharmacol*. 2003; 84: 105-108.
12. Aebi, HE. Catalase:In Bergmeyer HU. Ed. *Methods of enzymatic analysis*. Verlag Chemie, Weinhem, 1983; 273-286.
13. Misral, HP and Fridovich, I. The role of superoxide dismutase in the anti-oxidation of epinephrine and a simple assay for superoxide dismutase. *Jour Biol Chem* 1982; 247(10):3171-3175.
14. Varshney, R. and Kale, RK. Effect of Calmodulin Antagonist on Radiation-Induced Lipid Peroxidation in Microsomes. *International Journal of Radiation Biology*, 1990; 58, 733-743.
15. Vashney,R. and Kale, R.K. Effects of calmodulin Antagoniss. *Int. J Rad.Biol*.1990. 58:733-743
16. Habig, WH., Pabst, MJ., Jacoby, WB. Glutathion-stransferase. The first step in mercapturic acid formation. *Bio chem*. 1974. 249: 7130-7139
17. Metzger, BE. Long term outcomes in mothers diagnosed with gestational diabetes mellitus and their offsprings. *Clinical Obstetrics and Gynecology*. 2007; 50(4):972-979.
18. Seis H. Oxidative stress: Oxidants and antioxidants. *Experimental Physiology* 1997; 82(2): 291-295.
19. Anwar F, Latif F, Ashraf M, and Gilani AG. Moringa oleifera: A Food Plant with Multiple Medicinal Uses. *Phytother. Res*. 2007; 21: 17–25 Published online 6 November 2006 in Wiley InterScience ([www.interscience.wiley.com](http://www.interscience.wiley.com)).
20. Siddhuraju P and Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from different agroclimatic origin of Moringa oleifera. *Jour of Agric food chem*. 2003; 51, (18):2144-2155.

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21. Gomez-Zubeldia, MA, Arbues, J., Corrales, S; Millan, JS, and Nogales, AG. *Gynecologic Oncology* 2000; 86:250-258.
22. Yoshioka T, Motoyama H, Yamasaki F, Ando M, Takehara Y, and Yamasaki M. Lipid peroxidation and vitamin E levels during pregnancy in rats. *Biol Neonates* 1987; 52:223-231.
23. Kharb S. Total free radical trapping antioxidant potential in pre-eclampsia. *International Jour.of Gynecology and Obstetrics* 2000; 69(1) 23-26.
24. Guney M, Oral B, Demirin H, Karahan N, Mungan T, Delibas N. Protective effect of vitamin C and E against against endometrial damage and oxidative stress in fluoride intoxication. 2007; 34(5-6):467-475.
25. Singhal SS, Yallampalli C, Singhal J, Piper JT, Awasthi S. Purification and Gluthatione -S- Transferases of rat uterus. 1996; 11: 1271-1283.
26. Dube E, Vaillancourt C, Lafond J, Belkacemi L. Gestational diabetes affects placental-fetal development. In: *Pregnancy disorders and perinatal outcomes*. Ed. Julie Lafond, Cathy Vaillancourt; Belkam eBooks; Chapt. 6 pp88-95.
27. White V, Jawerbaum A, Sinner D, Pustovrh C, Capubianco E, Gonzales E. Oxidative stress and altered prostanoid production in the placenta of streptozotocin-induced diabetic rat. *Reprod Fertil Dev* 2002; 14:117-123.
28. Cederberg J, Siman CM, Erikson UJ. Combined treatment with vitamin E and vitamin decreases oxidative stress and improves fetal outcome in experimental diabetic pregnancy. *Pediatr Res* 2001; 49:755-762.
29. Dey P, Gupta P, Acharya NK, Rao SN, Ray S, Chakrabarty S. et al. Antioxidants and lipid peroxidation in gestational diabetes – a preliminary study. *Indian J Physiol. Pharmacol* 2008; 52 (2) : 149–156
30. Strain JJ, Disturbances of micronutrients and antioxidants status in diabetes. *Proceedings Nutrition Society* 1991;50: 591-604.