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Metformin Improves Reproductive Function in Diabetic Female Sprague-Dawley Rats

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Abstract: The female reproductive system provides several functions including ovulation, pregnancy and childbirth. The incidence of diabetes mellitus (DM) is increasing rapidly worldwide and it is associated with reproductive complications. Metformin is an oral hypoglycemic drug used in the management of DM. The present study investigates the effect of metformin in relation to reproductive function in alloxan-induced diabetic female rats. Rats were divided into four groups. Diabetes was induced with alloxan in the rats followed by a six week treatment with metformin. The pattern of the estrous cycle followed by the ova count were observed and recorded. Plasma hormonal levels of estradiol, progesterone, follicle stimulating hormone and luteinizing hormone were measured using Enzyme Linked Immunosorbent Assays (ELISA). Oxidative stress parameters and expression of estrogen receptors in the ovaries were determined. Results showed a significant increase ($p<0.05$) in the proestrus and estrus phases, with a significant decrease ($p<0.05$) in the diestrus phase in the treated diabetic group. A significant decrease ($p<0.05$) in the ova count, estrogen receptor expression and plasma hormone levels of catalase and glutathione were observed in all diabetic rats and these were significantly increased ($p<0.05$) following the administration of metformin. In conclusion, metformin improves some reproductive function in the diabetic state by increasing the expression of estrogen receptors, increasing the number of ova shed during ovulation and conferring foetal and maternal protective effects. Hence more consideration should be given to its possible use in the management of reproductive complications in DM.

KEYWORDS: Diabetes mellitus, Metformin, Reproductive function, Oestrogen receptor

1.0 Introduction

The female reproductive system provides several functions which include production of the ova (female egg) which are released through a process called ovulation. In humans, this event occurs when the Graafian follicles rupture and release the secondary oocytes. The process of ovulation is controlled by the hypothalamus of the brain through the release of hormones secreted in the anterior lobe of the pituitary gland (Marieb, 2013).

Diabetes mellitus (DM) is a condition in which the body does not produce enough, or does not properly respond to insulin, a hormone produced in the pancreas. Insulin enables cells to absorb glucose for ready conversion to energy (Rother *et al.*, 2007). In diabetes, the body either fails to properly respond to endogenously produced insulin, does not make enough insulin or both; this leads to glucose accumulation in the blood, resulting in various complications (Rother *et al.*, 2007). In 2000, according to the World Health Organization (WHO), at least 171 million people worldwide (2.8% of the population) suffered from DM. Its incidence is increasing rapidly, and it is estimated that by 2030, this number will almost double (Wild *et al.*, 2000). DM occurs throughout the world, but it is more common in the more developed countries (Wild *et al.*, 2000). The greatest increase is, however expected to occur in Asia and in Africa, where most patients will probably be found by 2030 (Wild *et al.*, 2000). DM has been associated with reproductive impairment in both men and women (David *et al.*, 2013). These include erectile dysfunction, in males, and

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spontaneous abortion and anovulation, in females (David *et al.*, 2013).

Metformin is the first-line drug for the treatment of Type II DM, particularly in overweight and obese people and those with normal kidney function (Bakkar *et al.*, 2005). It is the most popular anti-diabetic drug (Marina, 2007) and it is one of the only two oral anti-diabetics in the World Health Organization Model list of Essential Medicines (World Model List of Essential Medicines 2007). Metformin has recently become recognized as potentially beneficial for women with infertility and polycystic ovarian syndrome (PCOS) (Webster *et al.*, 2008). This syndrome appears to be related to insulin resistance, which is reversed by metformin, leading to successful and increased ovulation rates (Nestler *et al;* 1998). Though metformin is usually employed for management of hyperglycemia in Type II DM, the present study seeks to explore its hypoglycemic and also, non-hypoglycemic properties, and the effect(s) of these on the complications usually associated with reproductive functions in diabetic female rats.

2.0 Materials and Methods

2.1 Materials

2.1.1 Animals

Adult female Sprague Dawley rats weighing about 120-130g were obtained from the animal house of The College of Medicine University of Lagos. They were kept under a 12hr light: 12hr dark cycle and were allowed free access to feed and water throughout the experimental period. Animal identification was done in the Department of Cell Biology and Genetics, University of Lagos. All guidelines with the use and care of laboratory animals were strictly adhered to in accordance with the Institutional Animal Care Use Committee (IACUC, 2002).

2.1.2 Metformin

Metformin was a product of Sigma-Aldrich Inc, USA).

2.2 Methods

2.2.1 Induction of Diabetes

Diabetes was induced in the morning after fasting the animals overnight by a single intraperitoneal injection of 100 mg/kg of 10% w/v alloxan monohydrate (Sigma-Aldrich, Inc, USA) dissolved in distilled water. After 72 hours, blood glucose level of the dosed rats was checked and those with blood glucose level more than 200 mg/dl were considered, selected and used for the study.

2.2.2 Animal Grouping

Rats were divided into four groups (n =6) of (I) control (distilled water); (II) diabetic/non-treated (distilled water); (III) metformin/non-diabetic (100mg/kg daily) and (IV) metformin/diabetic (100mg/kg daily). Metformin was suspended in distilled water to yield a concentration of 25mg/ml. The dose selected was 100mg/kg daily and administered once a day (Choi et al; 2004). Administration lasted for 42 days.

2.2.3 Studies on Estrous Cycle

Animals were marked for identification. Vaginal smears were taken daily within the hours of 8am-10am daily. The estrous cycle was observed for 5 consecutive cycles using the pattern of cells in the obtained/collected smears with the aid of a light microscope (x 40). Rats with a regular 4 or 5 day estrous cycle were then selected for the study. Diabetic and non-diabetic rats were treated with metformin; and in the third week of treatment, the estrous cycle of the rats were observed (with continued drug administration) for another 5 cycles, using the method of Goldman *et al* (2007).

2.2.4 Studies on Ovulation

Following six weeks of drug treatment, normal and diabetic rats in estrus were

sacrificed, the uterine ducts removed and histological analysis conducted. Isolated uterine ducts were placed in a Petri dish containing normal saline; the ova shed were looked for under the light microscope (x100). The numbers

of ova shed were then counted and recorded appropriately (Duselis *et al*., 2005).

2.2.5. Determination of Hormones

Blood samples were centrifuged at 3000rpm for 15 minutes to obtain plasma for analysis. Plasma was kept in plain sample bottles and frozen at -20° C for hormonal assays. Assays were done using enzyme-linked-immunosorbent assay (ELISA) techniques. Unless otherwise stated, kits for biochemical assays were purchased from Enzo-life Sciences, Monobind Inc, USA, and Elabscience. Biochemical assays were carried out in strict compliance with manufacturers' instructions. Before the commencement of the assays, all reagents were brought to room temperature.

2.2.6 Determination of Antioxidant Parameters

The levels of malondialdehyde (MDA) was assayed by measuring the thiobarbituric acid (TBA) reactive species present in the test sample using the procedure of Vashney and Kale; (1990), and expressed as micromolar of malondialdehyde (MDA)/gramme of tissue. The activity of superoxide dismutase (SOD) was determined by its ability to inhibit the auto-oxidation of epinephrine at an absorbance of 480nm described by Fridovich; (1989). Catalase (CAT) activity was determined according by the method of Beers and Sizer (1952) as described by Usoh *et al;* (2005). The activity of glutathione peroxidase (GPx) was determined by the method described by Sedlak and Lindsay (1968). This is based on the development of a relatively stable yellow complex formed as a result of the reaction between Ellmans' reagent and free sulfhydryl groups.

2.2.7 Histological Examination

After removal, ovaries were weighed, fixed in 10% formalin, embedded in paraffin and 4μm sections were obtained. Sections were obtained at five different non-adjacent levels through the ovary with the third level being at the approximate midpoint. Sections were stained with hematoxylin and eosin. Follicular development was evaluated by counting the number of primary, secondary and tertiary follicles observed at each level. Follicular stage was categorized as previously described by Erickson *et al* (1986).

2.2.8 Immunochemistry

Paraffin sections (5μm) from five animals of each group were used. A standard immunohistochemical technique (avidin-biotin-peroxidase) was carried out to visualize estrogen receptors (ER) immunostaining intensity and distribution. The site of the bound enzyme was visualized and the counter-sections were counterstained with hematoxylin. A polyclonal rabbit anti-rat ERβ antibody was used to detect ERβ. Negative controls were obtained by replacing the primary antibody with non-immuno serum of equivalent concentrations (Coons *et al*., 1941).

2.2.9 Image Analysis

In order to quantify the ER mRNA expression, an Apex Microscope (Apex Researcher, Apex Microscope Limited, Chippenham, Wiltshire, UK) was connected to a computer using an Apex Minigrab. The number of golden stained nucleus per high-power field in sections from the control and treated animals were counted using an image processing program-Image j (NIH, 2007).

2.2.10 Statistical Analysis

Values expressed as mean ± standard error of mean (SEM) were analyzed by one-way ANOVA followed by Students' Newman-Keuls *post-hoc* test using the Graphpad Prism 6 software (2013). Differences were considered statistically significant at $p<0.05$.

3.0 Results

Figure 1 shows a significant decrease ($p<0.05$) in the percentage frequency of the proestrus phase in the diabetic group (12%) when compared to the control group (18%) and a significant increase ($p<0.05$) in the treated diabetic rats (16%)when compared to the untreated diabetic group. There was a significant

decrease ($p<0.05$) in the estrus phase of both diabetic groups (II-17% and IV-24%) when compared to the control group (30%), with a significant increase ($p<0.05$) in the treated diabetic group (24%) when compared to the diabetic untreated group (17%). The frequency of the metestrus phase showed insignificant changes ($p>0.05$) in the experimental groups (II-12%, III-13% and IV-10%) when compared to control (12%). The diestrus phase was significantly increased ($p<0.05$) in both diabetic groups (II-59% and IV-50%) when compared to control (40%), with a significant decrease ($p<0.05$) only in the treated diabetic group (50%) when compared to the untreated diabetic group. The diabetic untreated group (5.30±0.88) showed a significant increase ($p<0.05$) in the duration of the estrous cycle (Table 1) when compared to control (3.50±0.29); but changes in the treated diabetic group was not significant ($p>0.05$) when compared to the diabetic control.

Table 2 shows the effect of metformin on the ova count and fasting plasma levels of glucose and insulin in female diabetic rats. Ova counts in the untreated diabetic rats (4.33±0.65) showed a significant decrease ($p<0.05$) when compared to control (9.17±0.44); but this was significantly increased following administration of only metformin (6.50±0.61). There was a significant ($p<0.05$) increase in the plasma level of glucose in all the diabetic groups (II-277.75±8.58 mg/dl and IV-212.75±5.97 mg/dl) when compared to the control group (139.75±6.20 mg/dl). Fasting plasma level of insulin was also significantly decreased ($p<0.05$) in all the diabetic groups.

Table 3 shows the effect of metformin on the plasma levels of progesterone, estrogen, FSH and LH during the rat estrous cycle in female diabetic rats. There was a significant ($p<0.05$) increase in the plasma level of progesterone in the untreated diabetic group (2.43±0.15 ng/ml) and diabetic treated rats (group IV-2.55±0.19 ng/ml) when compared to the control group (0.58±0.09). The plasma level of estrogen showed a significant ($p<0.05$) decrease in the untreated diabetic group (100±1.83 pg/ml) with the rest of the experimental groups showing insignificant changes (III-136±13.1 pg/ml and IV-129±6.19 pg/ml). Reductions in the plasma level of LH, observed in all groups were only significant ($p<0.05$) in the diabetic groups (II-2.20±0.05 mIu/ml and IV-2.25±0.06 mIu/ml) when compared to control (I-3.12±0.21 mIu/ml). A similar decrease was observed in the plasma level of FSH in the experimental groups following treatment with metformin (II-1.02±0.01 mIu/ml and IV-1.05±0.01 mIu/ml)

The mean ER counts per HPF levels in control and experimental groups are shown in Figures 2-4. The mean ER in the control group was (27.00±1.00) as against experimental groups II (5.20±0.86), III (24.00±1.58) & IV (15.8±1.39). Significant reductions ($p<0.05$) in the diabetic and diabetic treated groups was observed when compared to the Control group with a significant ($p<0.05$) increase when the diabetic metformin treated groups was compared to the diabetic control.

Figures 5-8 show the effect of metformin on antioxidant enzymes (SOD, GPx and CAT activity) and lipid peroxidation (MDA concentration) in control and experimental rats. The GPx level (Figures 7-8) was significantly reduced ($p<0.05$) in the untreated diabetic rats (gp II-4.96±0.78 μ/mg) when compared to control (gpI-8.81±0.59 μ/mg). Non-significant ($p>0.05$) changes in the GPx level were observed in the rest of the experimental groups (III-8.83±4.26 μ/mg IV-& 8.67±0.91 μ/mg). GPx was significantly elevated ($p<0.05$) when the treated diabetic group (8.67±0.91 μ/mg) was compared to the untreated diabetic group (4.96±0.78 μ/mg). The CAT levels (fig 15-16) of experimental rats (groups II-1.97±0.59 μ/mg, III-2.99±0.39 μ/mg and IV-3.13±0.40 μ/mg) were significantly reduced ($p<0.05$) when compared to control (gpI-4.83±0.18 μ/mg). No significant change ($p>0.05$) was observed in the SOD activity in experimental groups (II-6.98±0.21 μ/mg, III-6.98±0.045 μ/mg and IV-6.55±0.065 μ/mg) when compared to control (gpI-6.35±0.004 μ/mg)

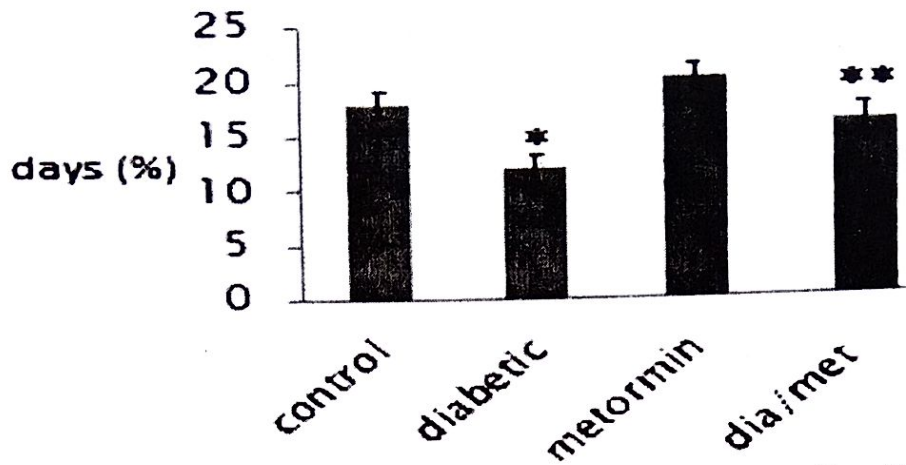

Figure 1: Proestrus stage of the estrous cycle of rats following treatment with metformin
*p<0.05 when compared to control group. ** denotes p<0.05 when compared to diabetic group

Table 1: Frequency and Duration of Estrous Cycle in Control and Experimental Rats

Groups	Cycles (no.)	Duration (days)
Control (Drug vehicle)	6.0	3.50±0.29
Diabetic (Drug vehicle)	3.9*	5.30±0.88*
Metformin (100mg/kg)	5.6	3.75±0.48
Dia/Met (100mg/kg)	4.4	4.70±0.67

* p<0.05 when compared to control

Table 2: Effect of metformin on ova count, plasma glucose and insulin levels in rats

Variable	Control (drug vehicle)	Diabetic (drug vehicle)	Metformin (100mg/kg)	Dia/Met (100mg/kg)
Ova Count	9.17±0.44	4.33±0.65*	9.00±0.33	6.50±0.61**
Fasting Plasma glc (mg/dl)	139.75±6.20	277.75±8.58*	130.84±5.11	212.75±5.97**
Fasting Plasma insulin (μIU/ml)	25.10±2.31	10.12±1.81*	23.53±6.20	9.75±1.14*
Glc:Insulin ratio	5.35	27.28*	5.56	21.82*

* p<0.05 when compared to control group
** p<0.05 when compared to diabetic group

Table 3: Effects of metformin on plasma levels of selected hormones of rats

Variable	Control (drug vehicle)	Diabetic (drug vehicle)	Metformin (100mg/kg)	Diabetic/Met. (100mg/kg)
FSH (mlu/ml)	1.70±0.10	1.02±0.01*	1.25±0.30*	1.05±0.01*
LH (mlu/ml)	3.12±0.21	2.20±0.05*	2.95±0.01	2.25±0.06*
Estrogen (pg/ml)	140.00±11.40	100.00±1.83*	136.00±13.1	129.00±6.19**
Progesterone (ng/ml)	0.58±0.09	2.43±0.15*	0.70±0.11	1.45±0.19**
Fasting Plasma glc (mg/dl)	139.75±6.20	277.75±8.58*	130.84±5.11	212.75±5.97**
Fasting Plasma insulin (μIU/ml)	25.10±2.31	10.12±1.81*	23.53±6.20	9.75±1.14*
Glc:Insulin ratio	5.35	27.28*	5.56	21.82*

* $p<0.05$ when compared to control; ** $p<0.05$ when compared to untreated diabetic group; FSH = Follicle Stimulating Hormone, LH= Luteinizing Hormone, Glc= Glucose

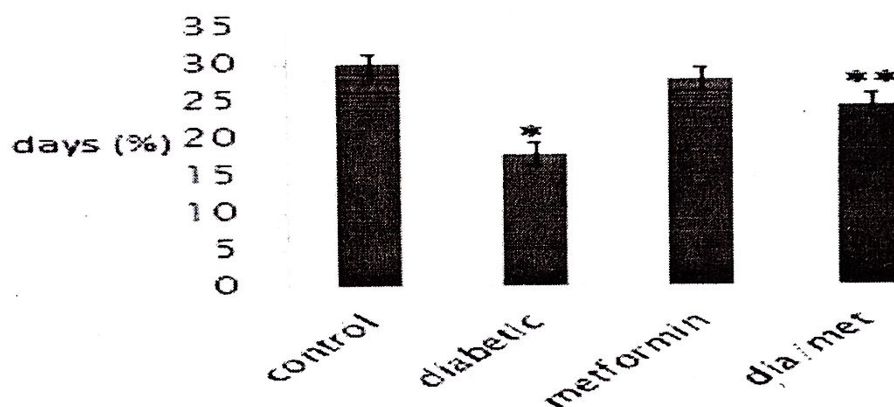

Figure 2: Frequency of the estrus stage of the estrous cycle in rats following treatment with metformin. * $p<0.05$ compared to control; ** $p<0.05$ compared to diabetic group

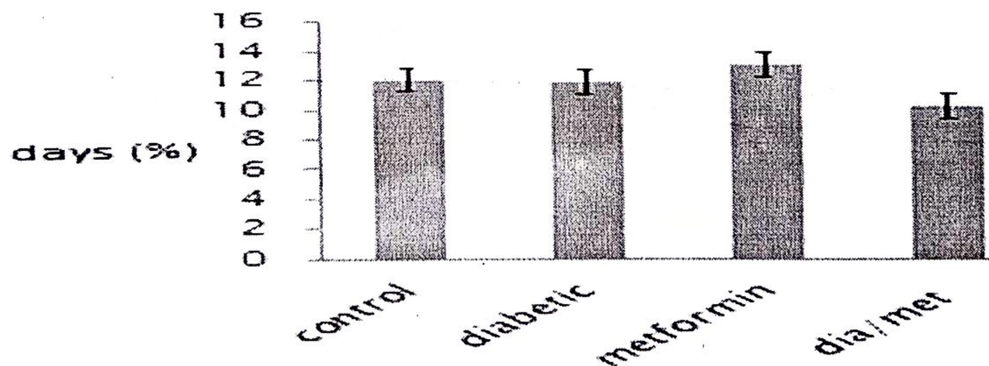

Figure 3: Frequency of the metestrus stage of the estrous cycle of rats following treatment with metformin. * $p<0.05$ compared to control

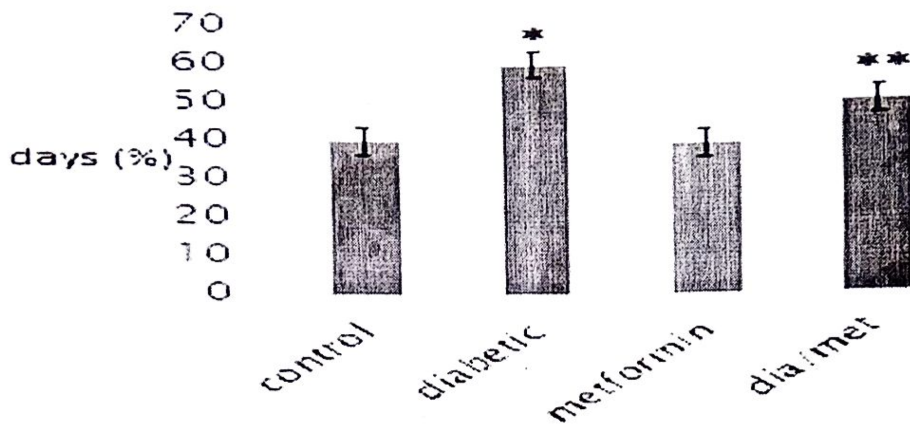

Figure 4: Frequency of the diestrus stage of the estrous cycle of rats following treatment with metformin. * $p < 0.05$ compared to control; ** $p<0.05$ compared to diabetic group

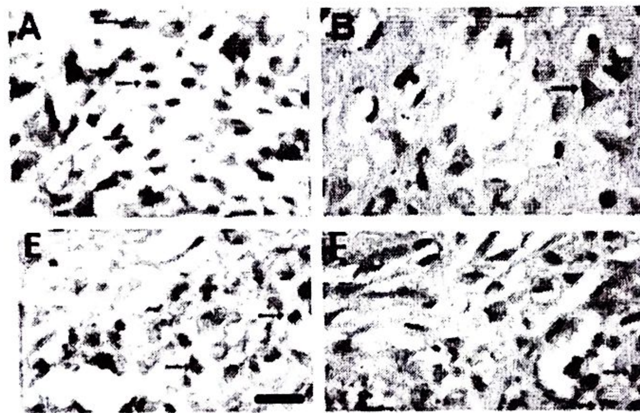

Figure 5: Immunohistological localization of Estrogen Receptor [ER] in the ovaries of rats; (A) ER of control rats (drug vehicle), (B) ER in diabetic rats (drug vehicle), (E) ER in non-diabetic treated rats (metformin-100 mg/kg); (F) ER in diabetic treated rats (metformin-100 mg/kg). Scale bar = 50 μm

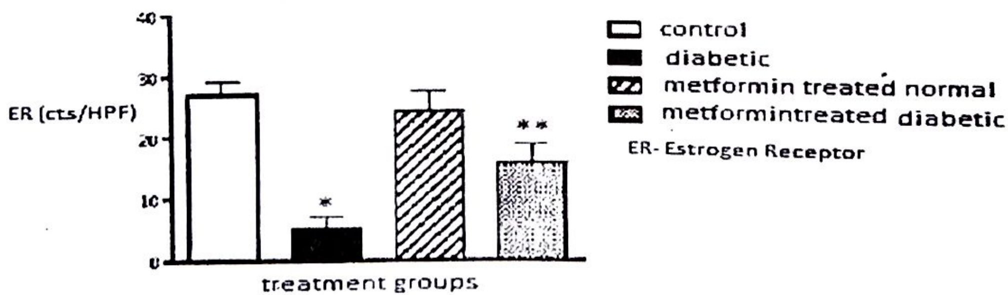

Figure 6: Effect of metformin on ER in diabetic rats. The counted cells per high power field (cts/HPF) observed in control, diabetic and metformin (100 mg/kg) treated normal and diabetic animals. Data was analysed using ANOVA with values of $p < 0.05$ considered statistically significant (*denotes $p < 0.05$ when compared to control group, ** denotes $p < 0.05$ when compared to the diabetic group)

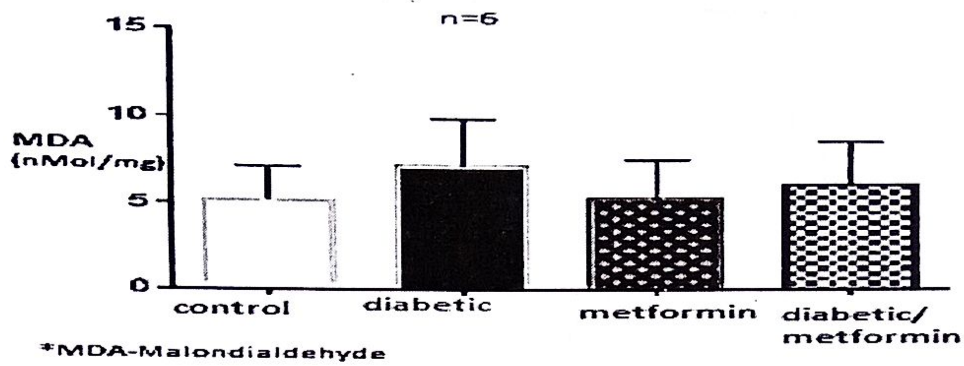

Figure 7: Effect of metformin on MDA in rats.
Data was analysed using ANOVA with $p<0.05$ considered as statistically significant

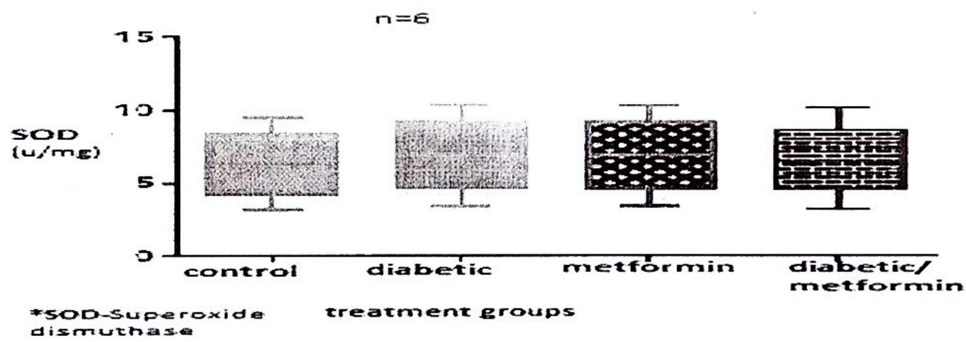

Figure 8: Effect of metformin on SOD of rats.
Data was analysed using ANOVA with $p<0.05$ considered as statistically significant.

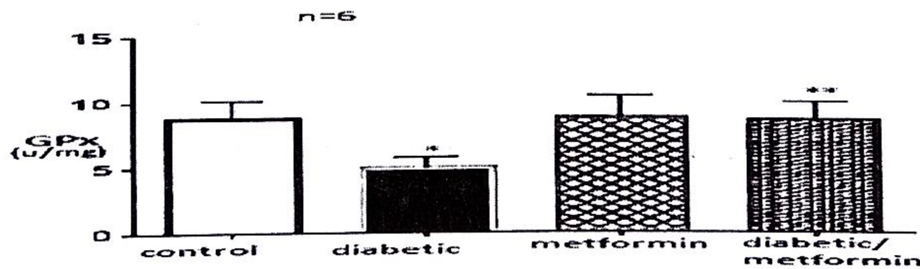

Figure 9: Effect of metformin on GPx on experimental rats.
Data was analysed using ANOVA. *significant when compared to control group. **significant when compared to diabetic group

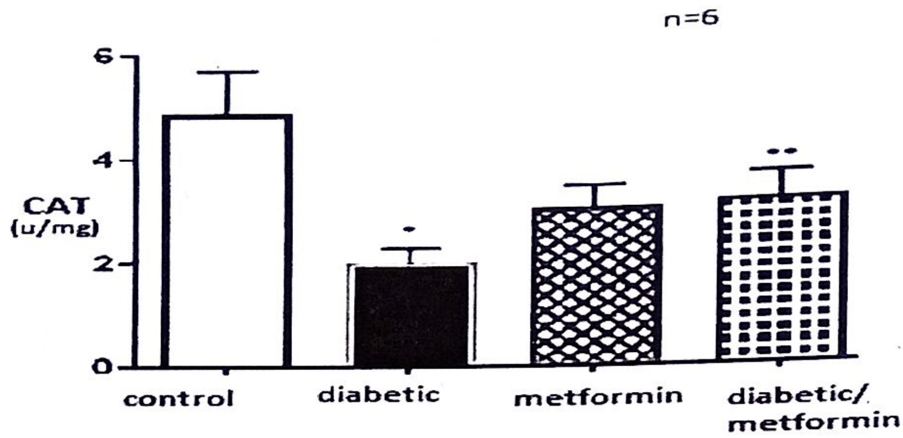

Figure 10: Effect of metformin on CAT in experimental rats.
Data was analysed using ANOVA; *significant when compared to control group; **significant when compared to diabetic group

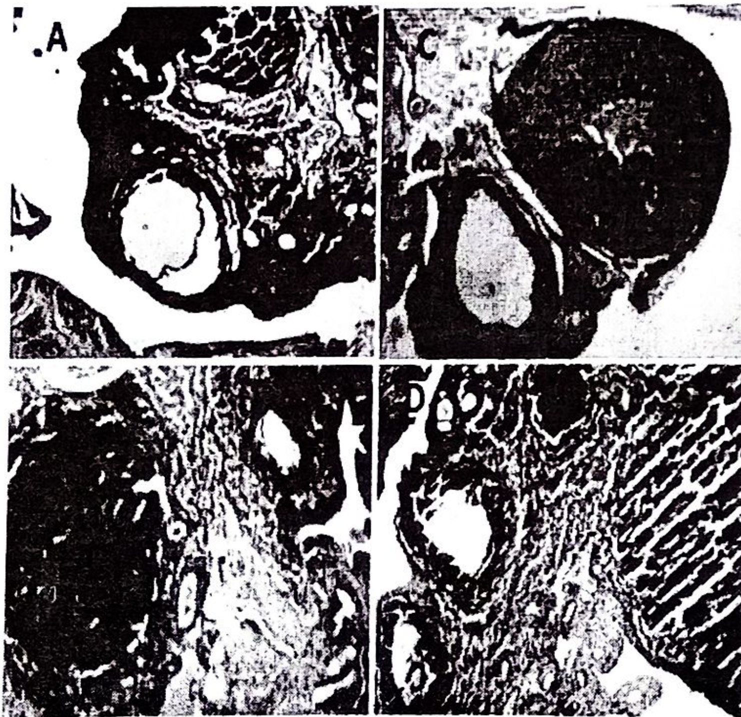

Figure 11: Cross section of the ovary (**H & E** ×40). **A**-Control (drug vehicle), B-Diabetic (drug vehicle), C-Metformin (100mg/kg), D-Diabetic/Metformin treated (100mg/kg), Corpus luteum Follicle Antrum Medulla

4.0 Discussion

The effect of metformin on the estrous cycle of the diabetic rat was studied. The normal rat model served to quickly identify the interactions and the diabetic models served to validate the same response expected when the drugs are used in treatment of DM. A significant predominance of the diestrus phase was noticed in the diabetic and diabetic treated groups. This caused a prolongation of the cycles and a subsequent increase in the period of time required to complete a cycle. This would translate to fewer cycles in a given period of time. This finding corresponds to that of Davis *et al* (1947), in which the estrous cycle was prolonged with irregularities in diabetic rats. Meanwhile, Anindita *et al.*, 2010, reported a shorter estrous cycle in diabetic rats and suggested that this was as a result of subtle alterations in the HPG-axis. The estrous cycle is largely controlled by hormones of the HPG-axis, mainly progesterone and estrogen, and it is disrupted by genetic, nutritional and endocrine factors (Iranloye and Bolarinwa; 2007). Several drugs can affect the estrous cycle by acting at the different levels of the hypothalamic-pituitary axis or at the ovarian level to inhibit ovulation. Ovulation in rats occurs from the beginning of proestrus to the end of estrus. There was a significant decrease in the ova count in the diabetic rats with a subsequent significant increase only following administration of metformin. This can be explained as a manifestation of the increase in the frequency of the proestrus and the estrus phases of the estrous cycle recorded in the treated diabetic group. Different studies (Tosca *et al.*, 2011, Rice *et al.*, 2009) have been conducted to determine if the effect of metformin on the ovary is direct or indirect; and even though the mechanism of action of metformin still remains unclear (Michael *et al.*, 2014), this study clearly shows that metformin improves on ovulation in diabetic rats. Estrogen plays a major role in ovarian follicular development and most of its effect is mediated by the transmembrane estrogen receptor (Chakrabothy and Roy, 2013).

Immunohistochemical studies performed at different time points of culture revealed that estrogen receptor beta (ERβ) was found exclusively in granulosa cell nuclei, regardless of follicular growth stage or culture conditions (Sandy and Johan; 2008). In granulosa cells of maturing follicles, estrogen is required for maximum FSH stimulation of CYP19 expression and estradiol synthesis, LH-receptor expression and LH responsiveness, antrum formation, gap-junction formation and prevention of atresia (Sandy and Johan; 2008). Therefore, the increase in the expression of ER recorded in this study would lead to a possible increase in follicular growth and oocyte maturation, sustenance of the oocyte and to the increase in ovulation observed in the present study.

The menstrual cycle is the regular and natural changes that occur in the uterus and ovaries that make pregnancy possible (Silverthorn and Unglamb, 2013). It involves interactions between the pituitary gland in the base of the brain, the follicles in the ovary and the lining of the uterus. The combination of estrogen, progesterone, FSH and LH serve to modulate and control the processes which occur in this cycle. The plasma levels of FSH and LH were decreased in all the experimental groups but this was only significant in the diabetic and rats. And even though the decreased levels observed in the diabetic rats were significant when compared to the FSH and LH levels in the control group, there was no significant change when the levels in the treated diabetic group were compared to the untreated diabetic group. These findings correspond to findings by Palomba *et al* (2010), who observed changes in the hormonal levels following the induction of ovulation with metformin. It was suggested that this was probably due to a direct action of the drug on the ovary (Palomba *et al.*, 2010). A significant decrease in FSH and LH levels was also reported in diabetic rats in studies by Djursing (1983). The decreased FSH and LH levels could be as a result of a possible feedback mechanism that could have been caused by the significantly increased progesterone levels recorded in the diabetic and diabetic treated rats. Also, estrogen in small amounts has a strong effect in inhibiting the production of both FSH and LH, and when progesterone is available, this inhibitory effect of estrogen is multiplied (Guyton and Hall, 2006). The feedback effect seems to operate

mainly directly on the anterior pituitary gland but to a lesser extent on the hypothalamus to decrease the secretion of GnRH by altering the frequency of the GnRH pulses (Guyton and Hall, 2006). This further explains a possible mechanism by which anovulation complicates DM. A decrease in plasma levels of LH can interfere with the LH-surge that is required for ovulation to occur. Without this initial pre-ovulatory surge of LH, ovulation will not occur. This can lead to anovulation, amenorrhea and infertility.

Reactive oxygen species (ROS) cause membrane damage to luteal cells (Sawada and Carlson; 1996). They also inhibit the transport of cholesterol to mitochondria with a subsequent reduction in the production of progesterone (Musicki *et al.*, 1994). The concentration of MDA is a direct evidence of toxic processes caused by free radicals and it is used to quantify the extent of lipid peroxidation (Hussain *et al.*, 2001). The result from the present set of experiments showed an insignificant decrease in MDA in the ovaries, following daily administration of both metformin for 42 days. SOD rapidly converts superoxide anion (O_2^-) to less dangerous H_2O_2. It has also been suggested that the accumulation of ROS and a decrease in SOD levels are involved in apoptotic cell death (Rueda *et al.*, 1995). In the present experiments, increasing levels of SOD (though not significant $p>0.05$) was recorded in the experimental groups. A significant increase in the levels of SOD would have suggested a possible response of the in-vivo antioxidant defense system to the rise in lipid peroxidation (exhibited by increase in MDA levels in the diabetic rats). It could also have been due to an enhancement of SOD activity by the direct effect of the administered metformin and gliclazide. This is similar to reports by Omotayo *et al* (2010), who observed a significant elevation of SOD in the pancreas of diabetic rats, but no significant changes following the administration of metformin. Catalase is important in the antioxidant defense against H_2O_2, but there are conflicting reports of decrease (Robertson *et al.*, 2003), increase (Bloch *et al.*, 2003), and no change in catalase activity in DM. CAT activity in the ovaries was significantly decreased in the diabetic control group compared to control. The treated diabetic rats also showed reduced catalase activity but with an up-regulation towards the non-diabetic control. CAT which inactivates H_2O_2, is an endogenous enzyme which needs to be replenished (Helmut, 1993). The implication of SOD up-regulation is that there could be a high turnover of H_2O_2, and its continued formation might overwhelm CAT activity. A significant rise was also recorded in the level of CAT and GPx in the diabetic rats following treatment. The combined effect of an elevation in the levels of both CAT and GPx would lead to an increase in intracellular antioxidant defense with a subsequent reduction in molecular damage caused by free radicals, especially in the diabetic state. These would suggest that metformin could maintain the cellular integrity of the ovaries in the diabetic state.

Estrogens and estrogen receptor modulators bind to ER to form discrete molecular complexes that exert pleiotropic tissue effects by modulating the expression of target genes (Joseph *et al.*, 2004). The ER is also capable of ligand-independent activity via a variety of intracellular signaling pathways, including the mitogen-activated protein kinase (MAPK) pathway. These signaling pathways exert their effects through the phosphorylation of the ER protein kinases, or indirectly through the regulation of cofactors bound to the ER. Hyper-activation of MAPK levels leads to its irreversible up-regulation (Oh *et al.*, 2001). A significant decrease in the expression of ER was observed in the diabetic groups when compared to control (non-diabetic); but there was also a significant increase in ER expression when the metformin treated diabetic rats were compared to the diabetic control. In this present study, following metformin administration in diabetic rats, a decrease in the elevated blood glucose levels was recorded. This could lead to the increase in the ER expression seen in the metformin treated diabetic group. In recent years, the 5-AMP activated kinase (AMPK) pathway has been identified as a potential target of antidiabetic therapies (Zou *et al.*, 2004). AMPK is a cellular protective mechanism that can exert antioxidant, anti-inflammatory and anti-apoptotic effects (Lee *et al.*, 2005). Metformin has also been shown to activate the AMPK via a LKB1-dependent mechanism

(Yong *et al.*, 2008). Insulin increases ER protein expression. It does this through the insulin-IGF-1 pathway. Metformin can also act by increasing insulin sensitivity and this can enhance the activity of insulin at the tissues. This could lead to an increase in the ER expression seen in this present study.

Conclusion

Findings from this study show that metformin increases ER expression and antioxidant enzyme activity in the ovary, but only in the diabetic state. The increase in ovarian ER expression and the increase in the frequency of phases of proestrus and estrus of the estrous cycle in the treated diabetic rats provide a possible mechanism of action of how metformin administration lead to the increase in ovulation recorded. An increase in the frequency of the diestrus phase of the estrous cycle with a subsequent rise in serum progesterone levels and a decrease in the serum levels of FSH and LH recorded is also suggestive of a possible mechanism by which anovulation complicates DM. These suggest that metformin can improve some reproductive function in the diabetic state. Hence more consideration should be given to the non-hypoglycemic effects of this drug and possible use in the management of reproductive complications associated with DM.

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