Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 5874 - 5895
Received 15 April 2021; Accepted 05 May 2021.

# Effect of Repaglinide and Metformin as Anti-Diabetic Drugs on Fertility and Sex Hormonal Levels of Male Albino Rats

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## Abstract

**Background** : The weakness of reproduction system in male that complaining with diabetes, by effects on the endocrine functions lead to depression of ejaculation, libido and erection in male, diabetes is associated to reduce ejaculated semen volume with decreased vitality and motility of the spermatozoa, with no change in sperm viscosity, the critical concern, as it is important to ensure diabetic individuals' reproductive health while on anti-diabetic medications. Therefore; this study has investigated the effect of repaglinide and metformin as anti-diabetic drugs on mating ratio and sex hormones levels of albino rats

**(2) Methods**: in this study induction T2DM in 28 male rats by injection of alloxan 3 doses of 120 mg/kg intra-peritoneal, and classified to 4 groups: 1-Control without any drug (positive control) 2-Treated by metformin (500 mg/kg) 3-Treated by replagnide (4 mg/kg) 4-Treated by metformin (500 mg/kg) and replagnide (4 mg/kg), and control groups without diabetes include 28 male rats, and classified into same groups of drugs administration, after 50 days of gavaging, choice two rats from each group for test the sexual performance and fertility by mating each male with two female rats, and sacrificing other animals and isolated serum sample and frozen at (-20) until to biological test (LH, FSH and testosterone) **(3) Results**: The percentage of mating was performed 100% in diabetic rats among 3 weeks while the diabetic male rats treated with metformin and animals treated with repaglinide complete the mating percentage in two weeks, whereas the diabetic rats treated with mixture of met+repa performed100% of mating rates in first week, The results revealed significant decrease ($P<0.05$) in mean of testosterone and luteinizing hormone in the diabetic compare with non-diabetic rats, but no significant differences ($P>0.05$) showed in other groups of study. The estimation of follicle stimulating hormone levels in study groups revealed significant decrease ($P<0.05$) in the animal of diabetic group compared to non-diabetic rats, and in the groups of animals treated by mixture of drugs (metformin and repaglinide) also observed significant elevation ($P<0.05$) in the diabetic rats compared to non-diabetic rats, while there was no significant differences ($P>0.05$) among other groups.**(4) conclusion:** Mixture of treatments metformin and repaglinide have very positive effect on diabetic animals and enhancement sexual activity and restore sexual hormones level.

**Keywords:** diabetes, repaglinide, metformine, sex hormones, fertility.

## Introduction

Hyperglycemic syndrome is one of the greatest public health, fears the modern societies, because the prevalence is rapidly increasing. In 21 century, the World Health Organization (WHO) reported that there were about 171 million people with DM, which represented a 60% increase relatively to 1995 (Alves *et al* ., 2013). Javanbakht *et al*., (2015) predicted to increase the present in 2030 there will be about

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300 million individuals affected by DM, which could represent a 39% increase comparatively to 2000, however the statistics that point to an overall 4.4% prevalence in the world population could be take too lightly since the factors known to be responsible for the disease development, such as obesity and lifestyle habits, may heighten these numbers.

A glance closer into fertility rates of modern societies reveals that the increased incidence of diabetes mellitus has been closely associated with falling birth rates and fertility, this is due to a disturbing increase of diabetic men in reproductive age. (Alves *et al*., 2013). Diagnosis of male infertility includes a physical examination, semen analysis, and hormonal tests, if warranted. Male infertility can result from a low sperm count, which means the testes have produced less sperm than normal. Difficulty sperm release from testes, or they might not be fully functional. Male infertility may also result from a number of factors including: underlying health conditions, retrograde ejaculation, environmental pollutants and lifestyle factors (Patel *et al*., 2018).

Certain diabetic complications can cause issues for men that contribute to infertility, in men both types of DM have long been recognized as major risk factors for sexual and reproductive dysfunction. This primarily includes impotence, erectile dysfunction (ED), ejaculatory (retrograde ejaculation) and orgasmic problems, as well as low desire (reduced libido), also impaired spermatogenesis that associated with DM (Chen *et al*., 2019)

Problems of male fertility may become more wide spread as diabetes rates rise, already the frequency of defective spermatogenesis and accompanying decreases in sperm parameters such as sperm count and motility, lifestyle factors have had a dramatic impact on general health and the capacity to procreate and increase type 2 DM which has led to the increased risk for infertility (Aboua *et al*.,2013).

The major classes of oral antidiabetic drugs include sulfonylureas, meglitinide, biguanide, thiazolidinedione (TZD), and dipeptidyl α-glucosidase inhibitors (Chaudhury *et al*., 2017), the vital target for the pharmacotherapy is to modify disease progression by decrease β-cell dysfunction, and long-term complications associated with hyperglycemia (Zhao, 2015)

Metformin is one of the oldest and the safest agents used in the treatment of DM, it from biguanide group of anti-diabetic medication and the first choice of recommended therapy for T2DM according to the International Diabetes Federation Global Guideline for DM (Inzucchi *et al*., 2012), its effects primarily by reducing hepatic glucose output through inhibition of gluconeogenesis and has a comparatively lesser effect increasing insulin sensitivity. Hence, unlike sulfonylureas, metformin is primarily an anti-hyperglycemic agent, does not cause hypoglycemia (Sacks *et al*., 2011).

Other important type of diabetic drugs is repaglinide, a carbamoylbenzoic acid derivative, that chemically is belong to the meglitinide class of insulin secretion agent, but unrelated to the sulfonylurea, it has a distinct binding site at the β-cell membrane. The clinical efficacy and tolerability of oral repaglinide in the treatment of patients with type 2 diabetes and provides an overview of its pharmacological properties, it is important stimulator for insulin secretion that lowers blood glucose by targeting early-phase insulin release. (Scott, 2012).

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This study was aimed to investigation about effect of repaglinide and metformin as anti-diabetic drugs on rats fertility by evaluate matting ratio and LH, FSH and testosterone levels.

## Methods

### Animals of Experiments

The study was conducted in laboratories of the University of Babylon / College of Sciences / Biology Department / Iraq, for the period from March to October 2020. Experiments were performed on eighty eight (88) rats (56 males and 32 females) albino rats (*Rattus rattus*) with body weight ranging from 200-250± gm and the age 8-14 weeks. The animals were ventilated and maintained at temperature 25 ºC. Animals were later adapted for experimental studies, and food and water were made available ad libitum.

### The occurrence of type 2 DM

The rats were given varied i.p. doses of alloxan to cause diabetes. (100,120,130 and150 mg / kg dissolved immediately in 0.5 mL normal saline) and different mode of dosing: single or multiple (dose each 24h), determined the dosage of 120 (3 doses) mg / kg body weight caused sustained hyperglycemia throughout experimental periods (Al-Joubori, 2013). After weekly measured of fastig blood glucose (FBG) and rats have more than 200 mg /dl were reflected diabetic occur and used in this study (Ganesh *et al*.,2010).

### Preparation of diabetic drugs and Detection the Animal Equivalent Dose (AED)

Anti-diabetic drug (Metformin 500 mg/kg) was obtained from local pharmacies under the name Glucophage and supplied by the company of a subsidiary of Merck Sanate (France). The other diabetic drug used in this study is Repaglinide (4mg/kg) was obtained from local pharmacies under the name Novonorm and supplied by the company of a subsidiary of Novo Nordisk (Denmark), then using the special equilibrium for determine the animal equivalent dose (AED) depend on method of Nair and Jacob, (2016), also after the insurance of the occurrences of type II DM. The treatment performed by dissolving in distilled water (DW) immediately and administrated orally by orogastric tube.

### The experimental design of study

The study included fifty six (56) of animals, after determined the best dose of alloxan (120 mg/kg by triple dose), fifty six of male rats was classified into two main groups, first group (28 rats) include 4 sub groups of male rats which treated by alloxan (DM inducer) each of them contain 7 rats :

1-Control without any drug (positive control)

2-Treated by metformin (500 mg/kg)

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3-Treated by replagnide (4 mg/kg)

4-Treated by metformin (500 mg/kg) and replagnide (4 mg/kg)

Second groups (28 rats) also include 4 sub groups but without alloxan treated ad also each of them contain 7 rats:

1-Control without any drug (negative control)

2-Treated by metformin (500 mg/kg)

3-Treated by replagnide (4 mg/kg)

4-Treated by metformin (500 mg/kg) and replagnide (4 mg/kg)

The weight of animals was taken every week by using electronic balance, also recorded the fasting blood glucose (FBG) by using glucometer, when complete the oral administration period (50 days) and after 24 hours of the last dose, 5 animals of each group were sacrificed after weighing and anesthetized by chloroform. The abdominal cavity was opened by a sharp scalpel the removed the testes and epididymis and put in petri dish containing the normal saline (glucose 5%), both left testis and epididymis used for the purpose of sperm parameters study such as sperm concentration sperm motility percent, sperm viability percent and the abnormal sperm morphology percent.

**Fertility and Sexual efficiency tests**

These tests occur by using 32 adults female rats, the remaining 2 male animals from each study groups, each one placed with two of the females in a special breeding cage , where the existence of vaginal plug was examined after the coupling for the purpose of sexual efficiency and fertility tests, and weekly monitoring to determine the time of conception.

**Blood Sampling**

The animals of all groups were sacrificed, after one day from the end of the experiment and blood was collected by heart puncture directly. The collected blood was centrifuged (3000 rpm for 20 minutes), the serum was collected for measuring hormones.

**Hormonal study**

**Measurement OF Luteinizing Hormone (LH)**

The measurement of LH levels was tested by using Elisa kit special for rats made by Bioassay Technology Laboratory (Chinese company)

**Principle of assay** The kit was an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Rat LH antibody. LH present in the sample is added and binds to antibodies coated on the wells. Then biotinylated Rat LH

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Antibody was added and binds to LH in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated LH antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Rat LH. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

**components**

**Table-1 LH kit components**

| **Apparatuses** | **Quantity** |
|---|---|
| Standard Solution (40mIU/ml) | 0.5ml x1 |
| Pre-coated ELISA Plate | 12 * 4 well strips x1 |
| Standard Diluent | 3ml x1 |
| Streptavidin-HRP | 3ml x1 |
| Stop Solution | 3ml x1 |
| Substrate Solution A | 3ml x1 |
| Substrate Solution B | 3ml x1 |
| Wash Buffer Concentrate (25x) | 20ml x1 |
| Biotinylated Rat LH Antibody | 1ml x1 |
| User Instruction | 1 |
| Plate Sealer | 2 pics |
| Zipper bag | 1 pics |

**Reagent Preparation**

1- All reagents were brought to room temperature before use.

2- The 120µl of the standard (40mIU/ml) with 120µl of standard diluent standard were reconstituted to generate a 20mIU/ml standard stock solution, and allowed the standard to sit for 15 mins with gentle agitation prior to making dilutions. Duplicate standard points were Prepared by serially diluting the standard stock solution (20mIU/ml) 1:2 with standard diluent to produce 10mIU/ml, 5mIU/ml, 2.5mIU/ml and 1.25mIU/ml solutions. Standard diluent serves as the zero standard(0 mIU/ml). Any remaining solution was frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

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**Table-2 dilution and standard solution of LH**

| | | |
|---|---|---|
| 20mIU/ml | Standard No.5 | 120µl Original Standard + 120µl Standard Diluent |
| 10mIU/ml | Standard No.4 | 120µl Standard No.5 + 120µl Standard Diluent |
| 5mIU/ml | Standard No.3 | 120µl Standard No.4 + 120µl Standard Diluent |
| 2.5mIU/ml | Standard No.2 | 120µl Standard No.3 + 120µl Standard Diluent |
| 1.25mIU/ml | Standard No.1 | 120µl Standard No.2 + 120µl Standard Diluent |

| Standard Concentration | Standard No.5 | Standard No.4 | Standard No.3 | Standard No.2 | Standard No.1 |
|---|---|---|---|---|---|
| 40mIU/ml | 20mIU/ml | 10mIU/ml | 5mIU/ml | 2.5mIU/ml | 1.25mIU/ml |

Twenty milliliter of Wash Buffer Concentrate 25x was diluted into deionized or distilled water to yield 500 ml of 1x Wash Buffer.

**Assay Procedure**

1- All reagents were prepared in the room temperature, standard solutions and samples as instructed.

2- The number of strips required for the assay was determined. The strips in the frames was inserted for use. The unused strips was stored at 2-8°C.

3- Fifty microliter of standard was added to standard well.

4- Forty microliter of sample was added to sample wells and 10µl anti-LH antibody was added to sample wells, then 50µl streptavidin-HRP was added to sample wells and standard wells. The well mixing was occur. The plate with a sealer was covered and incubated for 60 minutes at 37°C.

5- The sealer was removed and the plate was washed for 5 times with wash buffer. Wells were Soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash.

6- Fifty microliter of substrate solution A, and 50µl of substrate solution B were added to each well. The covered plate with a new sealer was incubated for 10 minutes at 37°C in the dark.

7- Fifty microliter of Stop Solution was added to each well, the blue color will change into yellow immediately.

8- The optical density (OD value) of each well was determined immediately by using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

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**Measurement of follicle stimulating hormone (FSH)**

The measurement of FSH levels was tested by using Elisa kit special for rats, made by Bioassay Technology Laboratory (Chinese company).

**Assay Principle** The kit was an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Rat FSH antibody. FSH present in the sample was added and binds to antibodies coated on the wells. Then biotinylated Rat FSH Antibody is added and binds to FSH in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated FSH antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Rat FSH. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

**Components**

| **Apparatuses** | **Quantity** |
|---|---|
| Standard Solution (64mIU/ml) | 0.5ml x1 |
| Pre-coated ELISA Plate | 12 * 4 well strips x1 |
| Standard Diluent | 3ml x1 |
| Streptavidin-HRP | 3ml x1 |
| Stop Solution | 3ml x1 |
| Substrate Solution A | 3ml x1 |
| Substrate Solution B | 3ml x1 |
| Wash Buffer Concentrate (25x) | 20ml x1 |
| Biotinylated Rat FSH Antibody | 1ml x1 |
| User Instruction | 1 |
| Plate Sealer | 2 pics |
| Zipper bag | 1 pics |

**Reagent Preparation**

1-All reagents were brought to room temperature before use.

2- The 120µl of the standard (64mIU/ml) with 120µl of standard diluent Standard were reconstituted to generate a 32mIU/ml standard stock solution , and allowed the standard to sit for 15 mins with gentle agitation prior to making dilutions. Duplicate standard points were prepared by serially diluting the standard stock solution (32mIU/ml) 1:2 with standard diluent to produce16 mIU/ml, 8mIU/ml, 4mIU/ml and 2mIU/ml solutions. Standard diluent serves as the zero standard (0 mIU/ml). Any remaining solution was frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

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**Table-3 Dilution and standard solution of FSH**

| | | |
|---|---|---|
| 32mIU/ml | Standard No.5 | 120µl Original Standard + 120µl Standard Diluent |
| 16mIU/ml | Standard No.4 | 120µl Standard No.5 + 120µl Standard Diluent |
| 8mIU/ml | Standard No.3 | 120µl Standard No.4 + 120µl Standard Diluent |
| 4mIU/ml | Standard No.2 | 120µl Standard No.3 + 120µl Standard Diluent |
| 2mIU/ml | Standard No.1 | 120µl Standard No.2 + 120µl Standard Diluent |

| Standard Concentration | Standard No.5 | Standard No.4 | Standard No.3 | Standard No.2 | Standard No.1 |
|---|---|---|---|---|---|
| 64mIU/ml | 32mIU/ml | 16mIU/ml | 8mIU/ml | 4mIU/ml | 2mIU/ml |

Twenty milliliter of Wash Buffer Concentrate 25x was diluted into deionized or distilled water to yield 500 ml of 1x Wash Buffer.

**Assay Procedure**

1-All reagents were prepared in the room temperature, standard solutions and samples as instructed.

2 - The number of strips required for the assay was determined. The strips in the frames was inserted for use. The unused strips was stored at 2-8°C.

3-Fifty microliter of standard was added to standard well.

4-Forty microliter of sample was added to sample wells and 10µl anti-FSH antibody was added to sample wells, then 50µl streptavidin-HRP was added to sample wells and standard wells. The well mixing was occur. The plate with a sealer was covered and incubated for 60 minutes at 37°C.

5- The sealer was removed and the plate was washed for 5 times with wash buffer. Wells were Soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash.

6-Fifty microliter of substrate solution A, and 50µl of substrate solution B were added to each well. The covered plate with a new sealer was incubated for 10 minutes at 37°C in the dark .

7-Fifty microliter of Stop Solution was added to each well, the blue color will change into yellow immediately.

8-The optical density (OD value) of each well was determined immediately by using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

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Received 15 April 2021; Accepted 05 May 2021.

**Measurement of Testosterone hormone (T)**

The measurement of Testosterone levels was tested by using Elisa kit special for rats, made by Bioassay Technology Laboratory (Chinese company).

**Assay Principle**

The kit was an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Rat T antibody. T present in the sample was added and binds to antibodies coated on the wells. Then biotinylated Rat T Antibody is added and binds to T in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated T antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Rat T. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

**Components**

| **Apparatuses** | **Quantity** |
|---|---|
| Standard Solution (3200ng/ml) | 0.5ml x1 |
| Pre-coated ELISA Plate | 12 * 4 well strips x1 |
| Standard Diluent | 3ml x1 |
| Streptavidin-HRP | 3ml x1 |
| Stop Solution | 3ml x1 |
| Substrate Solution A | 3ml x1 |
| Substrate Solution B | 3ml x1 |
| Wash Buffer Concentrate (25x) | 20ml x1 |
| Biotinylated Rat (T) Antibody | 1ml x1 |
| User Instruction | 1 |
| Plate Sealer | 2 pics |
| Zipper bag | 1 pics |

**Reagent Preparation**

1-All reagents were brought to room temperature before use.

2- The 120µl of the standard (3200ng/ml) with 120µl of standard diluent Standard were reconstituted to generate a 1600ng/ml standard stock solution, and allowed the standard to sit for 15 minutes with gentle agitation prior to making dilutions. Duplicate standard points were prepared by serially diluting the standard stock solution (1600ng/ml) 1:2 with standard diluent to produce 800ng/ml, 400ng/ml, 200ng/ml and 100ng/ml solutions. Standard diluent serves as the zero standard (0

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mIU/ml). Any remaining solution was frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

**Table-4 Dilution and standard solution testosterone**

| | | |
|---|---|---|
| 1600ng/ml | Standard No.5 | 120µl Original Standard + 120µl Standard Diluent |
| 800ng/ml | Standard No.4 | 120µl Standard No.5 + 120µl Standard Diluent |
| 400ng/ml | Standard No.3 | 120µl Standard No.4 + 120µl Standard Diluent |
| 200ng/ml | Standard No.2 | 120µl Standard No.3 + 120µl Standard Diluent |
| 100ng/ml | Standard No.1 | 120µl Standard No.2 + 120µl Standard Diluent |

| Standard Concentration | Standard No.5 | Standard No.4 | Standard No.3 | Standard No.2 | Standard No.1 |
|---|---|---|---|---|---|
| 3200ng/ml | 1600ng/ml | 800ng/ml | 400ng/ml | 200ng/ml | 100ng/ml |

Twenty milliliter of Wash Buffer Concentrate 25x was diluted into deionized or distilled water to yield 500 ml of 1x Wash Buffer.

**Assay Procedure**

1-All reagents were prepared in the room temperature, standard solutions and samples as instructed.

2-The number of strips required for the assay was determined. The strips in the frames was inserted for use. The unused strips was stored at 2-8°C.

3-Fifty microliter of standard was added to standard well.

4- Forty microliter of sample was added to sample wells and 10µl anti-T antibody was added to sample wells, then 50µl streptavidin-HRP was added to sample wells and standard wells. The well mixing was occurred. The plate with a sealer was covered and incubated for 60 minutes at 37°C.

5-The sealer was removed and the plate was washed for 5 times with wash buffer. Wells were Soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash.

6-Fifty microliter of substrate solution A, and 50µl of substrate solution B were added to each well. The covered plate with a new sealer was incubated for 10 minutes at 37°C in the dark .

7-Fifty microliter of Stop Solution was added to each well, the blue color will change into yellow immediately.

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8- The optical density (OD value) of each well was determined immediately by using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

## Results and Discussion

### Sexual efficiency

Sexual activity of studied groups include negative and positive control revealed in table-5, the mating ratio of diabetic animals recorded 25% in the third day, 25% in the seventh day of the first week, 25% in the fourth day of the second week and 25% in the second day of the third week when compared with non-diabetic animals in the negative control which recorded 50% in the second day and 25% showed for each of third and fourth days at the first week of sexual activity test, in conclusion the results showed that 100% of mating preferred in first week of non-diabetic groups, while the 100% of mating preferred in three weeks of diabetic groups. The results may be refer to the effect of complications of diabetes mellitus on rats sexual activity compared with non-diabetic groups, the high prevalence of sexual disorders amongst DM patients could be due to prolonged hyperglycemia that causes impairment of sexual functions by causing atherosclerosis, diabetic neuropathy, diabetes-induced endothelial dysfunction and endocrinological changes (Asefa *et al*., 2019).

**Table-5 changes in percentages of sexual mating for diabetic and non-diabetic groups**

| | **Diabetic group (Positive control)** | | | | | | | **Non-diabetic group (Negative control)** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D / W | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| **1** | | | **25%** | | | | **25%** | | **50%** | **25%** | **25%** | | | |
| **2** | | | | **25%** | | | | | | | | | | |
| **3** | | **25%** | | | | | | | | | | | | |

Other study showed impotence directly related to DM is much more frequent in diabetic than in non-diabetic patients (Hamdan and Al Matubsi, 2009), also Jesmin, *et al*. (2003) revealed the erectile dysfunction is a common complication of DM, and is mainly related to disturbed communication between vascular and neuronal systems due to either weakened blood circulation in penile tissue or impairment of neuronal stimulation. In addition to the vascular and neuronal disturbance related to erectile

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dysfunction in diabetic patients, there are other elements involved, including hormonal changes, chronic diseases, malnutrition, penile tissue infection and psychological influences (Hamdan and Al Matubsi, 2009).

Our results showed in table-6 the mating ratio of diabetic animals treated with metformin have 25% for each of third and fourth days in the first week and 25% in each of second and fifth days of the second week compared with non-diabetic animals administrated by the same treatment revealed 50% in the second day and 25% in each of third and fifth days of the first week, these results may be refer to the direct effect of diabetes disease on rats, while metformin improvement sexual efficacy in study groups, several studies showed the fertility in diabetic males and animal models is impaired (La Vignera *et al*., 2009), and that diabetic patients frequently seek for fertility treatments, pregnancy rates being reduced in mating ratio of diabetic male (Mulholland *et al*., 2011).

Such evidence further supports that something is wrong in the reproductive potential of these patients and that alterations at several parts of male reproductive system might have happened, metformin typically at the therapeutic dose, appears to be encouraging when considering its direct effect on semen quality and sperm function. Such effect may be due to the ability of metformin to reduce the oxidative damage and lipid peroxidation, enhance AMPK activity, and restore the normal levels of pituitary-gonadal hormones (Tavares *et al*., 2018); free radicals such as malondialdehyde cause oxidative stress are believed to disrupt the neuronal and vascular activities controlling penile erection (El-Latif, *et al*., 2006). Metformin is able to restore follicle-stimulating hormone, leutinizing hormone, and testosterone (Adaramoye, Lawal, 2014), and oral administration of metformin succeeded to restore testosterone level back to normal in diabetic rats which make as important reason for decrease fertility defect occur by diabetes complication (Ayuob, *et al*., 2015).

**Table-6 changes in percentages of sexual mating for groups treated by metformin**

| | **Diabetic group** | | | | | | | **Non-diabetic group** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D / W | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 1 | | | **25%** | **25%** | | | | | **50%** | **25%** | | **25%** | | |
| 2 | | **25%** | | | **25%** | | | | | | | | | |

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Sexual efficiency in the group of diabetic animals treated by repaglinide showed 50% in the second day, 25% in the sixth day of first week and 25% in the first day of second week, but in the group of non-diabetic animals treated by the same drug revealed 50% at the second day and 25% recorded for each third and fifth days at the first week (table-7), which may be refer to the effect of repaglinide on sexual activity of diabetic and non-diabetic rats, it stimulation increase insulin secretion and increase glucose uptake to release energy in all cells,, diabetes may affect male reproductive functions at multiple levels as a result of its effects on the endocrine control of spermatogenesis, steroidogenesis, sperm maturation, impairment of penile erection and ejaculation. A large number of studies both on diabetic men and experimental diabetic animals have been published on the impact of DM on male reproductive functions during the past few years (Jangir and Jian, 2014).

Meglitinides and sulfonylurease have the same effect on β-cell by biding on the sulfonylurease receptor (SURs), and stimulate insulin secretion (Grant and Graven, 2016), after induction of a diabetic through administration of streptozotocin, the overall fertility parameters were affected showing an increase on sperm shape abnormality and a reduction in sperm count as well as testis weight. Several studies have indicated the relationship between diabetes and sex hormones, and other studies refer to the effect of diabetes treatment on sexual efficiency (Tavares *et al*., 2018); The important function of repaglinide is stimulate insulin secretion, that target one of the main defects that characterizes type 2 diabetes (Stein *et al*., 2013); The insulin effects on the male reproductive function is well mirrored by the numerous reports, insulin action is extended to all the levels of the male reproductive system, from the hypothalamus pituitary gland axis (HPGA) to the testis, also having a role in male accessory organs, erectile and ejaculatory function and ultimately on sperm, as we will discuss in the following sections. Insulin was shown to interfere with the HPG axis, stimulating LH and FSH release in pituitary cell cultures (Adashi *et al*., 1981) and GnRH secretion and expression in hypothalamic neurons in culture (Burcelin *et al*., 2003).

**Table-7 changes in percentages of sexual mating for groups treated by repaglinide**

| | **Diabetic group** | | | | | | | **Non-diabetic group** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D / W | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 1 | | **50%** | | | | **25%** | | | **50%** | **25%** | | **25%** | | |
| 2 | **25%** | | | | | | | | | | | | | |

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 5874 - 5895
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The animals of both groups diabetic and non-diabetic treated by mixture of drugs metformin and repaglinide preformed the test of sexual activity in the first week, diabetic animals showed 50% in the third day and 25% in each of fourth and seventh days of the first week, non-diabetic animals also treated by mixture of drugs recorded 50% in the second day and 25% in each of third and fifth days for first week (table - 8), this results may be refer to the conjoint effect of treatments on sexual efficiency of diabetic rats by regulate glycemic disorder, which will reflected on all body cells, also enhancement the sexual performance of non-diabetic rats.

Lotti *et al*., (2021) demonstrated an association between hyperglycemic and the presence of hypogonadism, decreased normal sperm morphology and erectile dysfunction in men lead to infertility problems, also explored the association between metabolic syndrom and prostatic abnormalities of infertile men. Male fertility is a case that can be influenced by metabolic syndrome which include several mechanisms obesity, dyslipidemia, hypertension, and diabetes mellitus. Endocrine system dysregulation, scrotal temperature elevation, oxidative stress, and alteration of the erectile and ejaculatory functions are well recognized metabolic syndrome consequences that can impair sperm production and function, ultimately affecting male fertility (Martins *et al*., 2019).

Fang *et al*., (2014) study the comparable effectiveness of repaglinide to metformin in reducing glycaemic variability, enhancing insulin sensitivity and ameliorating β-cell function. Nonetheless, these compounds also seem to affect the sperm, in one of the few studies performed, besides sulfonylureas, Al-kuraishy and Al-Gareeb (2016) concluded metformin leads to significant reduction in testosterone levels, sex drive and induction of low testosterone-induced erectile dysfunction, whereas; sulfonylurea leads to significant elevation in testosterone levels, sex drive and erectile function; Attia *et al*., (2009) showed obesity induced an increase in the number of sperm abnormalities and a decrease in the sperm concentration and motility which was rescued by metformin administration. In obese patients, metformin treatment improves sperm concentration and motility in the same way as observed in obese rats (Yan *et al*., 2015).

**Table-8 changes in percentages of sexual mating for groups treated by mixture treatment metformin and repaglinide**

| | **Diabetic group** | | | | | | | **Non-diabetic group** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D / W | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |

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Received 15 April 2021; Accepted 05 May 2021.

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | 50% | 25% | | | 25% | | 50% | 25% | | 25% | | |

**Hormonal assay**

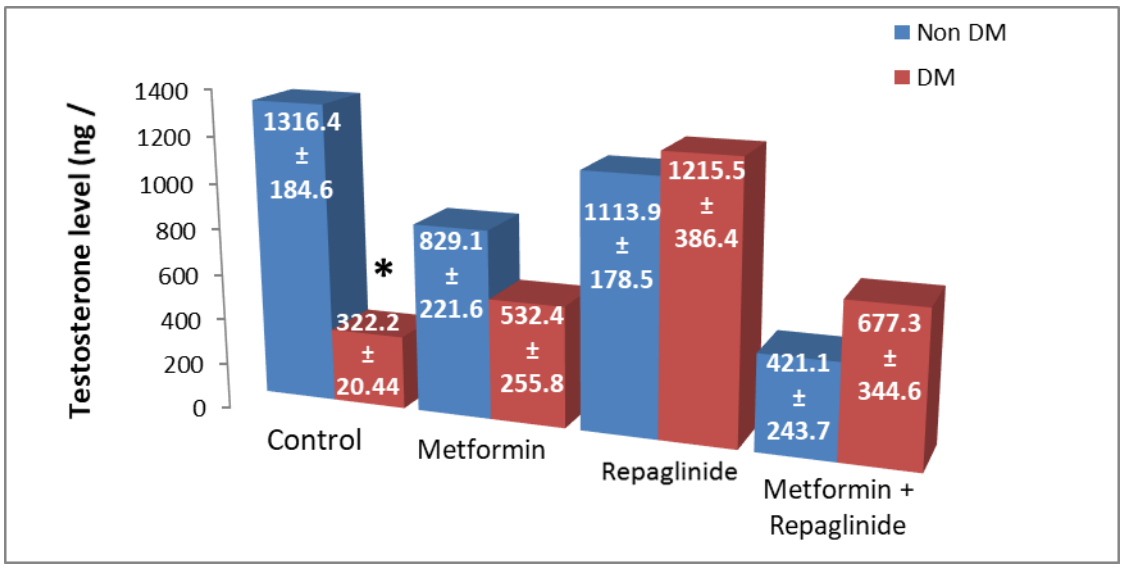


**Figure-1 Testosterone levels**
**significant differences at (P<0.05) (mean± S.D.)**

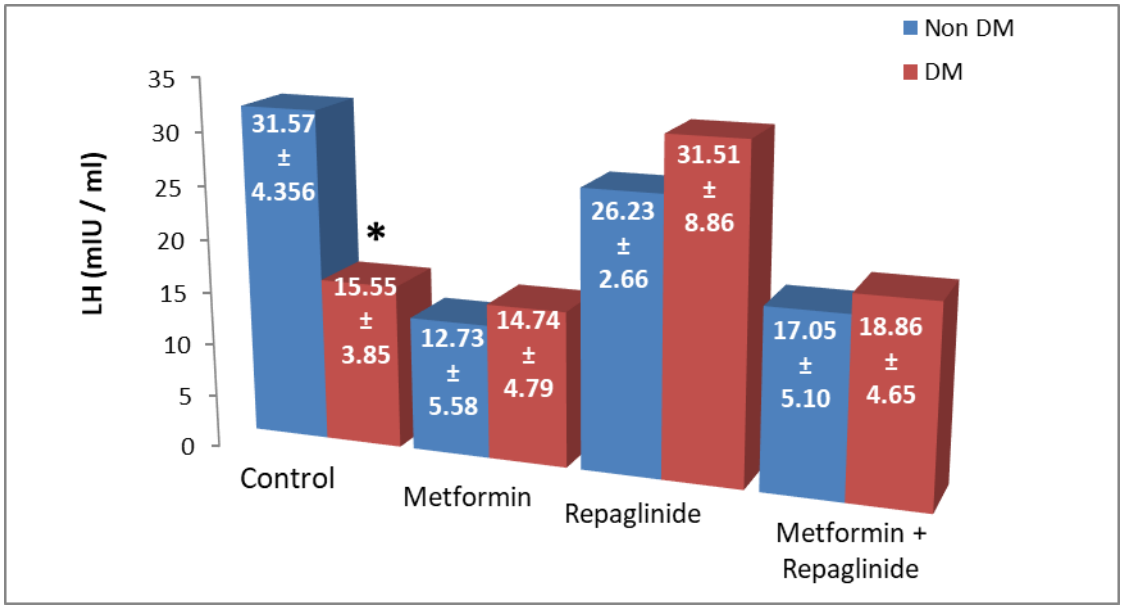


**Figure-2 Luteinizing hormone levels**
**significant differences at (P<0.05) (mean± S.D.)**

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 5874 - 5895
Received 15 April 2021; Accepted 05 May 2021.

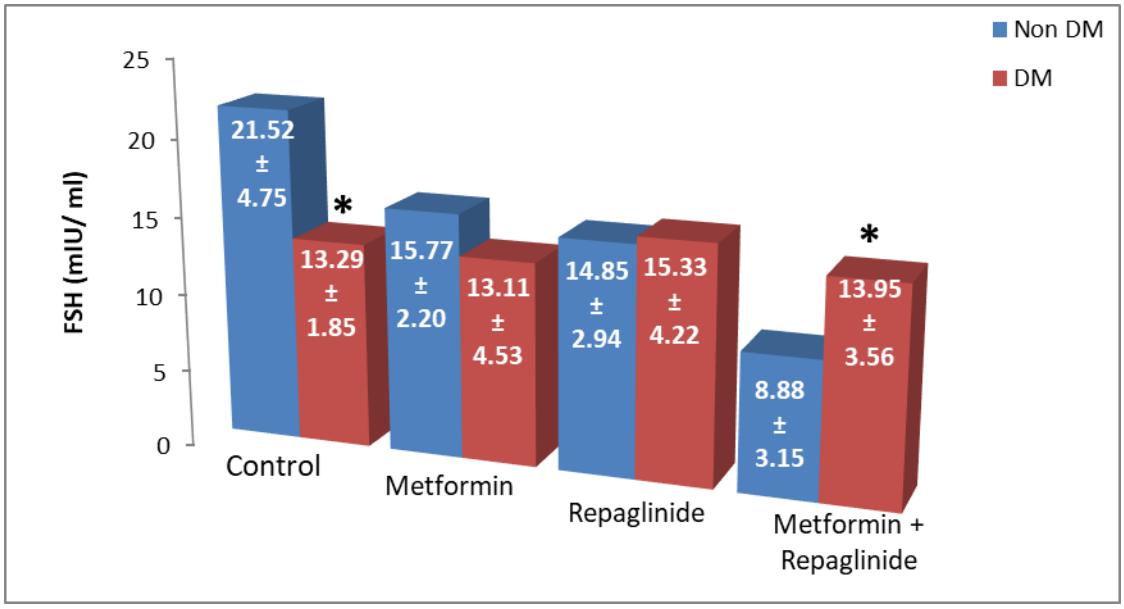


**Figure-3 Follicle Stimulating Hormone (FSH) levels significant differences at (P<0.05) (mean± S.D.)**

Figure-1 revealed significant decrease (P<0.05) of testosterone level in diabetic compared to non-diabetic rats, also results showed significant reduction (P<0.05) of luteinizing hormone in diabetic animals compare with non-diabetic (figure-2), and figure-3 revealed significant reduction (P<0.05) of follicle stimulating hormone in diabetic control group compare with non-diabetic.

May be refer to the effect of diabetes that caused oxidative stress in diabetic animals, the reduction of LH, FSH and testosterone may be resulted from oxidative stress cause hypothalamus damage, that effect on secretion of gonadotrophin-releasing hormone (GnRH) level that led to release LH and FSH (Ikpeme *et al*., 2016), other study concluded, LH and FSH levels decrease in diabetes mellitus type2 in both sexes (Hussein and Al-Qaisi, 2012). Other medical studies revealed low levels of LH and FSH, in addition to low testosterone levels in 25% of men of diabetes mellitus type 2 (Basu *et al*., 2012), other studies revealed reduction of LH and FSH in 33% of hypogonadism patients (Dhindsa *et al*., 2004).

Elabbady *et al*., (2016) mention patients of T2DM inclined to have low testosterone level, other study revealed the risk of type 2 diabetes in men can't associated with higher testosterone level, also reverse connection development in the relation between testosterone insufficiency and threat by diabetes type 2 in men (Yao *et al*., 2018).

Pituitary hormones managed function of testes primarily, spermatogenesis regulated by follicle-stimulating hormone, and Leydig cell function under control of luteinizing hormone. Diabetes cause reductions in the serum levels of prolactin FSH, LH, and growth hormone (Jelodar *et al*, 2010). Changes in LH and FSH caused lowering of testosterone level in diabetics, that commonest form hypogonadotropin (gonadal disorder) (Tripathy *et al*., 2003). Other study reported low testosterone and normal LH levels in diabetics (Ando *et al*.,1984); whereas, but other found that

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 5874 - 5895
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subjects with diabetic neuropathy had small level of testosterone associated with great LH and FSH levels (Ali *et al*.,1993).

Our results revealed no significant differences (P>0.05) between diabetic and non-diabetic rats, in testosterone, LH and FSH levels in groups of animals treated by metformin, and other treated by repaglinide, (figure 1, 2, 3), and groups of rats treated by mixture of drugs (metformin and repaglinide) revealed no significant differences (P>0.05) between diabetic and non-diabetic, in testosterone and LH levels, but revealed significant elevation (P<0.05) of FSH level in diabetic compared to non-diabetic rats, that may be refer to the enhancement effect of drugs on diabetic rat for regulated hormone level; and removal significant differences.

Mohsen *et al*., (2016) concluded disorders in sex hormones are associated with insulin resistance and development of type 2, also revealed the testosterone levels decreased significantly in male and increased significantly in female of patients group than control groups; Hyperglycemia can generate oxidative stress and reactive oxygen species (ROS) by several pathways (Ramalho-Santos *et al*., 2008). Oxidative stress and ROS from these pathways are associated with male infertility, it can induce a decrease in testosterone levels by damage hypothalamus pituitary gland axis, changes in seminiferous tubule structure, and spermatogenesis failure (Alahmar, 2019). High ROS concentration in semen has been demonstrated in 30%–40% of male infertility (Agarwal *et al*., 2014). Metformin has been shown to lower the risk of diabetes related complications, cardiovascular disease, stroke, reduces oxidative stress and restores antioxidant reserve (Esteghamati, *et al*., 2013). Metformin action is achieved mainly through the inhibition of mitochondrial electron transport chain complex I, which leads to an increase of AMP:ATP ratio, that will then trigger AMPK, switching off the anabolic process (Melnik and Schmitz, 2014), the main target of metformin is the liver, it can also act on other tissues/organs, including the reproductive ones (Tavares *et al*., 2018).

Metformin have the ability of to reduce oxidative stress and lipid peroxidation, enhance 5'-AMP activated protein kinase activity, and restore the normal levels of pituitary-gonadal hormones (Banihani, 2016). Younas *et al*., (2021) concluded that both metformin and repaglinide have similar anti-hyperglycemic effects, repaglinide can be prescribed as an alternative drug to metformin in patients diabetes mellitus type 2. Meglitinides are usually used in combination with metformin, a thiazolidinedione or insulin, although they can be used as monotherapy (Bianchi *et al*., 2018).

Clinical studies have found that, in addition to low testosterone levels, 25% of males with type 2 diabetes also present with low levels of LH and FSH (Basu *et al*., 2012); Previous investigations have found that 33% of patients with hypogonadism also had LH and FSH levels that were significantly reduced (Dhindsa *et al*., 2004). Repaglinide used for utilizes as monotherapy or in combination with metformin or other antidiabetic agents. Its short duration of action and administration previous to

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 5874 - 5895
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each meal makes it an ideal treatment for type 2 diabetic patients with a flexible lifestyle (Guardado-Mendoza, 2013); Repaglinide monotherapy has been shown to give the same improvement in glycemic control as glimepiride or glyburide in 12-month studies in type 2 diabetic patients (Marbury *et al*., 1999). Rates of hypoglycemia with repaglinide were compared to those seen in comparator trials versus glipizide and less than those versus glyburide (Cambell, 2010).

Repaglinide treatment is more beneficial for decreasing glucose variability in patients with type 2 diabetes by increase insulin secretion and decrease tissues injury after reduction blood glucose levels. These smaller glucose fluctuations are associated with decreased levels of inflammation and oxidative stress markers (Yamazaki *et al*., 2014). Treatment with repaglinide offers an additional benefit to type 2 diabetic patients, improving the parameters of oxidative stress. Thus repaglinide appears to be an effective drug in the treatment of type 2 diabetes either as monotherapy or in combination with metformin (Tankova *et al*., 2003).

## Conclusion

1-There is significant differences between diabetic and non-diabetic rats in sexual hormones level.

2- repaglinide enrichment sexual activity,

3-Repaglinide enhancement sexual hormones levels for diabetic animals

4-Mixture of treatments metformin and repaglinide have very positive effect on diabetic animals and enhancement sexual activity and restore sexual hormones level.

## Recommendation

1-Study the effect of repaglinide on semen parameters in-vitro.

2-Study the reduction effect of repaglinide on oxidative stress.

3-Histological study for repaglinide effects on testes and epidydimal tissues**.**

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