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Repaglinide and metformin (diabetic treatments) enhance sexual efficiency

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***Abstract*---**Diabetes mellitus type 2 (DMT2) is one of the modern societies' highest public health threats. For a while, it was believed that DM had no impact on male reproductive function; however, new research has cast doubt on that assumption. To find out whether repaglinide and metformin may improve sperm motility and testosterone levels in diabetic and non-diabetic albino rats, researchers in this study used these two drugs to treat diabetes. Methods: Alloxan injections at three dosages of 120 mg/kg intraperitoneal produced type 2 diabetes in male rats. Experimental rats were classified into two main groups. The first group included four subgroups of male rats treated with alloxan (DM inducer). Each subgroup contained seven rats "1. Control without any treatment (positive control), 2. treated by 500 mg/kg metformin, 3. treated by 4 mg/kg repaglinide, and 4. treated by 500 mg/kg metformin and 4 mg/kg repaglinide". The second group also includes four subgroups but without alloxan treated. Each subgroup has seven rats; all are categorized in the same initial group and receive identical treatment dosages. After Fifty days of administration and weekly tests for weight and fasting blood glucose level, then sacrificing the animals and test

progressive motility of epididymal sperm, blood sera were also taken to test testosterone levels. Results: The results demonstrated substantial variations (P<0.05) between final and initial body weight in all studied subgroups, except the diabetic rats treated with repaglinide and those treated with a combination of treatments. Also, the results revealed significant elevation (P<0.05) of testosterone in diabetic rats treated with repaglinide compared with groups of diabetic rats, diabetic rats treated with metformin and diabetic rats treated with a combination of treatments. It revealed a substantial rise (P<0.05) of testosterone in non-diabetic rats and group of non-diabetic rats treated with repaglinide compared to groups of non-diabetic rats treated with metformin and others treated by a combination of treatments in progressive motility percent. The study demonstrated a substantial reduction (P<0.05) in diabetic rats relative to diabetic rats treated with metformin, repaglinide, and metformin and repaglinide combination. It demonstrated a significant increase (P0.05) in sperm progressive motility in non-diabetic rats and a group of non-diabetic rats treated with repaglinide compared to non-diabetic rats treated with metformin and a group of non-diabetic rats treated with a combination of treatments. Conclusion: Repaglinide increases body weight compared with metformin, and repaglinide enhances the testosterone level in diabetic rats. It enhances the progressive motility content of sperm in diabetes and non-diabetic individuals relative to metformin. Also, the therapies combined with repaglinide and metformin increased progressive motility content in the diabetes group relative to the diabetic group.

***Keywords*---**diabetes, repaglinide, metformin, progressive motility, testosterone.

Introduction

Diabetes mellitus (DM) is a chronic metabolic turmoil distinguished by continuously increased blood glucose rates of more than 126 mg/dl and random plasma glucose readings of more than 200 mg/dl, raising the risk of serious and long-term health repercussions (Khawandanah, 2019). A deeper look at contemporary society's fertility patterns indicates that the rising prevalence of diabetes mellitus has been linked to lower fertility and birth rates. This rise is attributed to a disturbing rise in the number of diabetic males of reproductive age(Alves *et al.*, 2013).

Sulfonylureas, biguanide, thiazolidinedione, meglitinide, and dipeptidyl-glucosidase inhibitors are the most frequent oral anti-diabetic medications(Chaudhury *et al.*, 2017). Metformin is one of the most well-known and well-tolerated diabetes medications. This anti-diabetic drug, which belongs to the biguanide family, is recommended as the primary treatment for patients with type 2 diabetes by the IDFGG "International Diabetes Federation Global Guideline" for DM(Inzucche *et al.*, 2012). Its main purpose is to suppress gluconeogenesis, which reduces hepatic glucose synthesis and has a considerably

lower impact on improving insulin sensitivity. Metformin is mainly an anti-hyperglycemic medicine that might not produce hypoglycemia, despite the presence of sulfonylureas (Sacks *et al.*, 2011).

Another significant diabetes drug is repaglinide, a carbamoylbenzoic acid derivative corresponding to the meglitinide family of insulin secretion agents but unrelated to sulfonylureas. It binds to a specific location on the β-cell membrane. The pharmacological features of oral repaglinide are summarized based on its clinical effectiveness and tolerability in treating type 2 diabetes patients. It is an essential insulin secretion activator that lowers blood sugar by concentrating on early-phase insulin production(Scotte, 2012). The impact of metformin and repaglinide as anti-diabetic medicines on the fertility of lab rats is investigated in this research. The research measured epidydimal sperm progressive motility and testosterone levels in the blood.

Materials and Methods

Induction of T2DM

The rats were given 100, 120, 130, and 150 mg/kg i.p doses of alloxan dissolved instantly in 0.5 ml normal saline in single or multiple dosing modes (24h each) to induce diabetes (Al-Joubori, 2013). Each dose of alloxan for each rat was calculated based on the ratio and proportionality, according to the following equation(Erhirhie *et al.*, 2014):

$$\text{Alloxan dose} = \frac{120mg \times rat\ weight}{1000\text{gm}} \quad (1)$$

The glucometer was used to assess the FBG “fasting blood glucose” level of fasting rats every week and rats with FBG>200mg/dl were designated diabetic and employed in the current investigation(Ganesh *et al.*, 2010).

Diabetic drugs preparation and Animal Equivalent Dose Detection

Metformin (500 mg/kg), an anti-diabetic medicine, was bought from a local pharmacy under the brand name “Glucophage” and provided by Merck Sanate, a Merck subsidiary (France). Repaglinide (4 mg/kg) was the other diabetic medication utilized in this investigation. It was purchased from local pharmacy under the brand name “Novonorm” and provided by a subsidiary of “Novo Nordisk (Denmark)”. The equivalent animal dosage (AED) was computed determined by the ratio and proportionality after the occurrences of T2DM were insured. Initially, the dose was calculated for 1000 grams, and then it was converted in the same way to the weight of the used rat, depending on the following equilibrium(Erhirhie et al., 2014):

$$X = \frac{HED \times 1000\text{gm}}{human\ body\ weight} \quad (2)$$

$$\text{AED} = \frac{Animal\ body\ weight \times 1000\text{gm}}{X} \quad (3)$$

The result is then multiplied by factor 6.2(Nair and Jacob, 2016). The drug was administered orally by an orogastric tube after being instantly dissolved in distilled water (DW).

Experimental design

Fifty-six male rats were assigned to two equal groups; diabetic and healthy. Each group was allocated to 4 equal subgroups and treated as follows: without treatment, metformin-treated (500 mg/kg po), repaglinide-treated (4mg/kg po), and combination-treated subgroup. The treatment was extended for 50 days. Blood glucose level and body weight were measured weekly. At the end of treatment, animals were anesthetized by chloroform and sacrificed. Epidydimal were examined after weighing, and a sharp scalpel opened the abdominal cavity. After that, the epididymis is removed and placed in a petri dish containing normal saline. Sperm characteristics such as sperm progressive motility percent were studied using the epididymis. Blood sera were isolated and kept at -20^{0}C (Varley *et al.*, 1991) for testosterone assessment.

Blood sampling

All animals were sacrificed one day after the experiment ended, and blood was obtained via direct heart puncture. For 20 minutes, the blood was centrifuged at 3000 rpm. The serum was taken to determine hormone levels(AL-Saily, 2018).

The percent of sperm motility

After being removed, the left epididymis was weighed. After putting it in a 1 ml normal saline, it was sliced into a tiny piece with a sharp scalpel to release the sperm. A drop of the solution was put on a clean glassslide and examined under a 400X power microscope after fully mixed. Using Equation 4, a drop of epididymis solution was produced and deposited on the slide instantly to estimate the proportion of motile sperm in 10 random fields for every class.

$$\%\text{SpermMotility} = \frac{\text{Number of motile sperms}}{\text{Total sperms}} x100 \qquad (4)$$

According to WHO (WHO, 2010), sperm motility is categorized into three primary classifications; non-progressive motility sperm, progressive motility sperm, and immotile sperms.

Measurement of testosterone hormone (T):

Testosterone rates were determined by a “Bioassay Technology Laboratory Elisa” kit designed specifically for rats (China).

Testosterone hormone assay principle

An ELISA “Enzyme-Linked Immunosorbent Assay” kit was used. The plates have been pre-coated with a “Rat T” antibody. The letter “T” was added to the sample and bound to antibodies on the wells. The sample is then inoculated with the

biotinylated “Rat T” antibody, which binds to “T.” Then, “streptavidin-HRP” was added, which binds to the biotinylated “T” antibody. Following incubation, unbound “Streptavidin-HRP” has washed away during a washing phase. The substrate solution is then poured after that. The hue changes in direct proportion to the amount of “Rat T.” By applying an acidic stop solution, the reaction is halted, and the absorbance at 450 nm was measured.

Components

Table 1 presents the apparatuses and quantities used in the current research.

Table 1
Apparatuses and quantities

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

Reagent preparation

All reagents were brought to room temperature before use. To make a 1600 ng/ml standard stock solution, 120 l of the standard (3200 ng/ml) were reconstituted with 120 l of the standard diluent. The standard was permitted to sit for 15 minutes with gentle agitation before diluting. Duplicate standard points were made by diluting the “standard stock solution” (1600 ng/ml) 1:2 with standard diluent to get solutions at 800ng/ml, 400ng/ml, 200ng/ml, and 100ng/ml. Standard diluent (0 mIU/ml) was utilized as the zero standard. Any residual solution was kept at -20°C for a month and then used. Table 2 shows the suggested dilutions of standard solutions. 20 ml of wash buffer concentrate 25x was diluted into distilled or deionized water to provide 500 ml of 1x wash buffer.

Table 2. Standard solution and dilution testosterone

Concentration (ng/ml)	Standard No. (STN.)	Mixture with Standard Diluent (SD)
1600	5	120µl Original Standard + 120µl SD
800	4	120µl STN.5 + 120µl SD
400	3	120µl STN.4 + 120µl SD
200	2	120µl STN.3 + 120µl SD

100	1	120µl STN.2 + 120µl SD

Standard Conc. (ng/ml)	STN.5	STN.4	STN.3	STN.2	STN.1
3200	1600	800	400	200	100

Assay procedure

All reagents were produced at ambient temperature. The standard solutions and samples were made according to the directions. The number of strips needed for the test was calculated. The strips were put into the frames for usage. The strips that were not utilized were kept at 2 to 8°C. The standard well was filled with fifty microliters of the standard. Sample wells were filled with forty microliters of the sample and ten "anti-T" antibody microliters. After that, 50 µl streptavidin-HRP was applied to the sample and control wells. The mixing of the well has taken place. The plate was sealed with a sealer and incubated at 37°C for 60 minutes. After removing the sealant, the plate was washed five times with wash buffer. For each wash, the wells were submerged in at least 0.35 ml wash buffer for 30 to 60 seconds. Substrate solution A was 50 microliters, while substrate solution B was 50 µl. The sealed plate was incubated in the dark for 10 minutes at 37°C with a fresh sealer. Each well added 50 microliters of stop solution. The blue color will become yellow very instantly. Every well's optical density (OD value) was measured utilizing a microplate reader setted in 450 nm within 10 min. after injecting the stop solution.

Results and Discussion

Detection of alloxan doses

The effect of alloxan doses on fasting blood glucose levels is shown in Table 3. Hyperglycemia and diabetes mellitus were not caused by single doses of 100, 120, and 130 mg/kg body weight. The single dose of 150 mg/kg body weight caused severe hyperglycemia after two weeks, and 100% mortality of experimental animals was observed. The three 120 mg/kg body weight dosages resulted in long-term hyperglycemia over the study's duration. According to these findings, three doses of 120 mg /kg body weight were the dependent dosage in this research induction of diabetic Mellitus.

Table 3
Effect of different alloxan doses on fasting blood glucose levels of experimental animals

Dose (mg/kg) body weight	No. of animals	Dose type	Fasting blood glucose (mg/dl) (mean ± SE)			%Death
			Before induction	After induction	After 2nd week of induction	
100	7	SingleDose	96.3 ± 2.724	93.8 ± 2.24	93.1 ± 2.63	--
120	7		99.1 ± 3.88	92.12 ± 3.44	95.2 ± 4.081	--

130	7		100 ± 4.162	93.2 ± 4.812	99.5 ± 4.680	--
150	7		91.1 ± 4.284	473.5± 11.23	Died	100
120	28	Multiple (3 doses)	111.25 ± 5.041	264.18 ± 20.107	316.15 ± 16.150	--

The high doses of alloxan can destroy the β-cells (Masiello, 2006), so the death might be attributed to the full destruction of β-cells and the sharp deficiency in insulin level, which causes un-tolerated hyperglycemia. These results are inconsistent with other studies that used 150 mg/kg i.p. of alloxan to induce DM (Etuk and Muhammed, 2010). Also, Tanquilut *(Tanquilut et al., 2009)* used 200 mg/kg i.p. to induce DM in mice, while Al-Joboury (Al-Joubori, 2013) used alloxan intravenously at a 100 mg/kg dosage induce DM in rabbits.

These inconsistent results might be attributed to the differences in species, race, lab conditions, and injection route. For example, Abu Abeeleh (Abeeleh *et al.*, 2009) showed that using the same dose of diabetogen (streptozotocin) in two rats strains gave different results. The doses can differ according to this factor.

An optimal alloxan dose for rat diabetes induction research is still pending. Lower dosages may result in auto-reversion to a normal condition without medical treatment, but greater amounts induce toxicity and loss of experimental animals (Chougale *et al.*, 2007). Otherwise, alloxan causes diabetes by producing a selective cytotoxic effect on pancreatic cells and the formation of free radicals (Macdonald Ighodaro *et al.*, 2017). In diabetic rats, activity levels of antioxidant enzymes decreased, demonstrating the damaging influence of free radicals produced due to alloxan exposure (Resmi *et al.*, 2006).

Bodyweight

Table 4 compares the initial and final body weights in all the investigated groups. The positive control group, treated with alloxan and induced, exhibited a substantial reduction in final body weight (P>0.05) compared to initial body weight. In contrast, the rats in the negative control group, who had not been given diabetic injections, gained significantly more weight than they had at the beginning of the experiment (P>0.05). In diabetic rats, this finding was accepted since a drop in body weight is widely recognized as one of the most significant diagnostic symptoms of diabetes (ADA, 2017).

The production and storage of carbs, lipids, and proteins are stimulated by insulin, a potent anabolic hormone. This is accomplished by boosting the intake of amino acids, glucose, and fatty acids into cells and raising the expression and the activity of enzymes that catalyze lipid, glycogen, and protein synthesis. Insulin also prevents the degradation and release into the circulation of other hormones (Saltiel and Kahn, 2001).

So, reducing insulin sensitivity in diabetic animals may be one of the causative aspects of losing weight relative to increasing body weight in normal animals

(non-diabetic). Furthermore, diabetes causes an inability to retain fat and protein, as well as the breakdown of existing fat and protein stores (Ravi *et al.*, 2005), resulting in a reduction in body weight. The accelerated breakdown of tissue protein in diabetic rats leads to a loss in body weight (Ganesh et al., 2010).

Table 4
Bodyweight changes in experimental rats

Groups	Bodyweight (mean ± SE)		Sig differences p-value (0.05)
	Initial Bodyweight (gm)	Final Bodyweight (gm)	
1-Positive control (DM)	236.8 ± 10.876	176.7 ± 3.023	0.005*
2- DM + Metformin	225.0 ± 2.088	202.20 ± 1.911	0.003*
3- DM + Repaglinide	227.0 ± 3.189	229.60 ± 0.815	0.465
4- DM + Repaglinide + Metformin	226.6 ± 3.321	221.20 ± 1.603	0.154
5- Negative control	219.4 ± 2.925	242.4 ± 0.666	0.001*
6- Metformin treatment	222.60 ± 1.636	206.2 ± 0.722	0.02*
7- Repaglinide treatment	217.6 ± 3.365	251.4 ± 1.603	0.001*
8-Repaglinide + Metformin treatment	210.2 ± 3.806	213.6 ± 1.294	0.841

*****significant differences at (P<0.05) (mean± SE.)

Metformin treatment resulted in a substantial decrease in final body weight in diabetic animals (P<0.05). The group of non-diabetics that is additionally treated with metformin demonstrated a substantial drop in final body weight (P<0.05). These findings may pertain to the weight gain in diabetic animals as a consequence of the disease. It might also relate to the direct impact of metformin on body weight. A statistically substantial decline in the metformin group was ascribed by Malone (Malone, 2005) to a non-significant change in the sulfonylurea group. Several mechanisms explain metformin's weight-loss and health-promoting benefits. Further investigation of these mechanisms may be critical in identifying potential pharmacological targets for obesity and other metabolic diseases linked with ageing (Yerevanian and Soukas, 2019).

Diabetic animals treated with repaglinide exhibited no substantial variations ("P<0.05") between final and initial body weight. Non-diabetic animals treated with repaglinide showed a substantial weight gain (P>0.05) compared to the control group, suggesting that the drug's impact on the normal body weight of diabetic rats may be considerable. Another research indicated that the comparison between sulfonylureas and repaglinide caused comparable weight gain (Bolen *et al.*, 2007). Repaglinide treatment increases weight gain, although it does so at a slower rate than glibenclamide medication by boosting beta-cell insulin production. This activity stimulates glucose, amino acid, and fatty acid absorption into cells, as well as lipid and protein synthesis (Guardado-Mendoza *et al.*, 2013). Older well-controlled studies found that sulfonylurea medication resulted in an average increase in body weight in those with T2DM and that metformin therapy resulted in a comparable advancement in glycemic control (Lebovitz and Melander, 2015).

The result revealed no considerable variation (P>0.05) between the final and initial body weight in the diabetic animals administrated by metformin and repaglinide combination treatments. In the group of non-diabetic animals treated with the combination of treatments, no variations (P>0.05) in final and initial body weight were observed. It may refer to the effect of both drugs on body weight balance in the study groups. Metformin has been studied as a weight-loss prevention strategy in various diseases (Yerevanian and Soukas, 2019). According to another research, Metformin administration to diabetic rats resulted in increased drug concentrations in the cerebrospinal fluid. Metformin may be able to alter the hypothalamus by crossing the blood-brain barrier, according to the study. Metformin may also help overweight people lose weight by having a multilayer influence on neuronal appetite pathways and peripheral fat metabolism (Stevanovic *et al.*, 2012).

Metformin's ability to blunt weight gain, as observed with thiazolidinediones, sulfonylureas, and repaglinide, is likely due to these factors (Malin and Kashyap, 2014). Patients with type 2 diabetes on repaglinide were shown to gain weight when the medicine was taken with metformin, even when the two were taken separately (Moses *et al.*, 1999).

Testosterone levels in diabetic and non-diabetic rats

The results revealed a significant elevation (P<0.05) of testosterone in diabetic rats treated with repaglinide compared with groups of diabetic rats treated with metformin and the combination of treatments (Figure 1). This finding pertains to the impact of repaglinide that regulates blood glucose level and physiological function of hormonal secretion and may decrease oxidative stress compared to other study groups.

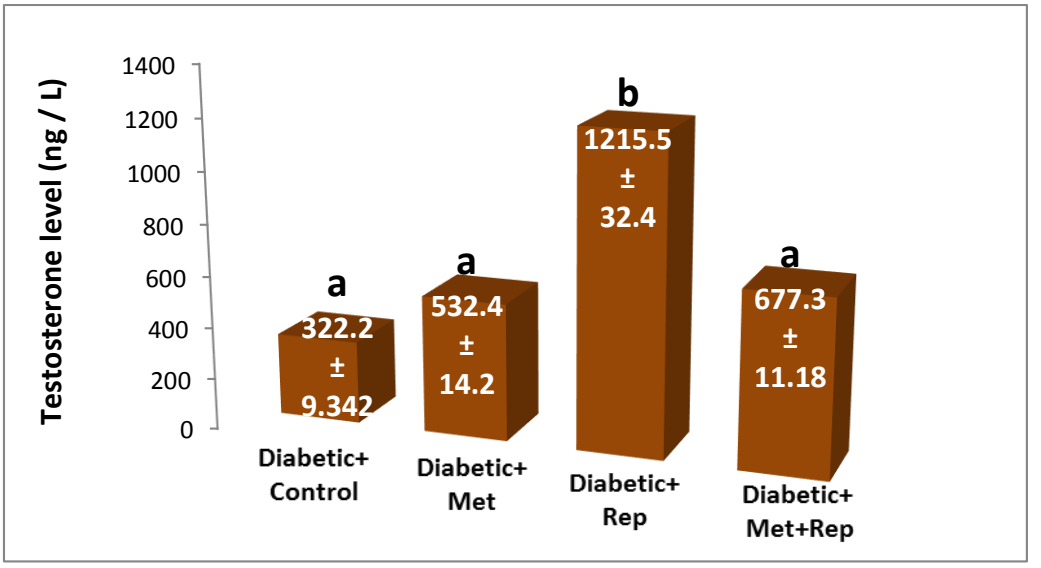

Figure 1. Testosterone levels of diabetic rats. Different letters demonstrated considerable variations at P<0.05 (mean±SE.)

A decrease in offspring, a decrease in Leydig cells population, the loss of epididymal sperm content, reduced spermatogenesis, and serum LH have all been reported during diabetes mellitus (Brüning *et al.*, 2000). Repaglinide improves glycaemic control in type 2 diabetic patients through its physiological mode action to reduce blood glucose levels. Type 2 diabetes individuals may benefit from improved oxidative stress measures while using repaglinide. Repaglinide, as a monotherapy or in combination with metformin, seems to be a functional drug in the treatment of T2DM (Tankova *et al.*, 2003).

Testosterone levels are associated negatively with blood sugar status in males, indicating its involvement in developing T2DM. Furthermore, low testosterone blood levels are more closely associated with diabetes and obesity, resulting in sexual dysfunction, physical fatigue, and changes in mood (Mattack *et al.*, 2015).

Insulin has been shown to partly or totally prevent testosterone and ABP levels from dropping. The epididymis of diabetic rats showed secretory activity and concentrating capacity, indicating that insulin plays a role in sperm maturation (Singh *et al.*, 2009). Sulfonylurea in type 2 diabetes raises testosterone, sexual desire, and erectile function significantly (Al-Kuraishy and Al-Gareeb, 2016). According to the findings, metformin decreases testosterone levels, sex desire, and erectile dysfunction in men with low testosterone.

Figure 2 demonstrated considerable rise ($P<0.05$) of testosterone in non-diabetic rats and rats given with repaglinide compared to groups of rats given with metformin and a combination of treatments. The testosterone levels in the animals treated with a combination of therapies were significantly lower ($P<0.05$) than in the other research groups, as shown in Figure 2. In non-diabetic rats treated with metformin and a combination of therapies, this drop may reflect repaglinide's enhancing impact, perhaps decreasing oxidative stress.

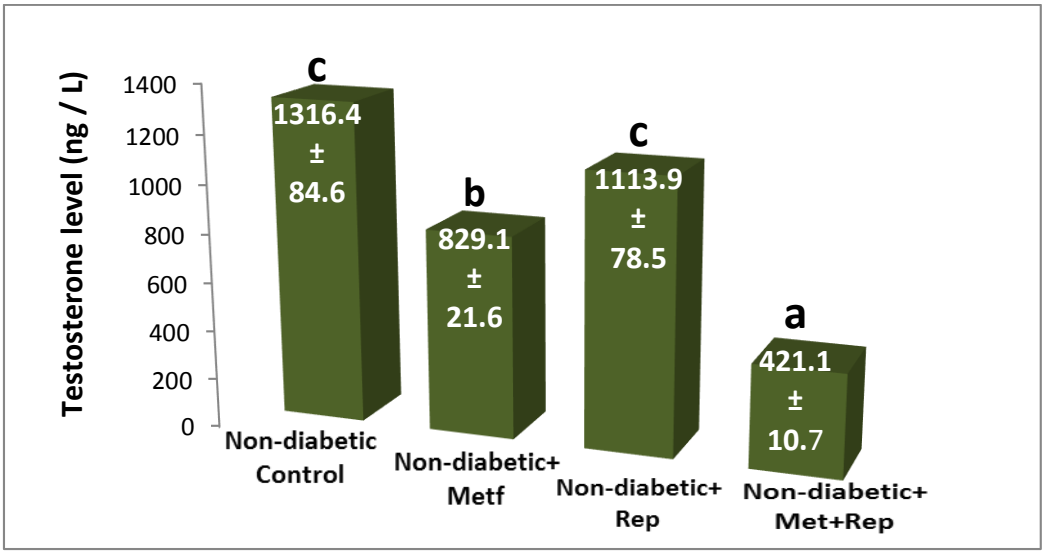

Figure 2. Testosterone levels of non-diabetic rats. Different letters demonstrated considerable variations at $P<0.05$ (mean± SE.)

Valsamakis (Valsamakis *et al.*, 2013) highlight the influence of diabetes medication on testosterone levels. Metformin dramatically decreases total testosterone by decreasing Cytochrome P450-C17a in the liver, a crucial enzyme in TT production and lowers LH hormone secretion. Metformin's detrimental impact on LH levels is another name for this phenomenon. After just a few days of usage, metformin rapidly reduces LH-stimulated testosterone (Kurzthaler *et al.*, 2014). Metformin decreases total testosterone and free testosterone in healthy men and raises sex hormone-binding globulin in males on a short-term basis (Shegem *et al.*, 2002). No significant variations in the mean blood LH and FSH levels were found between patients and controls by Ramkishanjat (RamkishanJat *et al.*, 2014).

Metformin enhances the efficacy of in vitro fertilization and is regarded as a supplemental medicine in assisted reproductive technologies. Also, metformin administration positively impacts spermatogenesis and steroidogenesis in males with metabolic disorders type 2 DM patients. Thus metformin treatment signals potential application to enhance male reproductive functioning and fertility (Shpakov, 2021).

Progressive motility percent

Figure 3 confirmed a substantial decrease ("P<0.05") of sperm progressive motility percent for diabetic rats relative to diabetic rats treated with metformin, repaglinide, and treatments. The findings also demonstrated a substantial drop ("P<0.05") in sperm progressive motility content in diabetic rats treated with metformin relative to other groups. No major changes (P<0.05) were observed between the diabetic rat groups treated with repaglinide and the combination of medications.

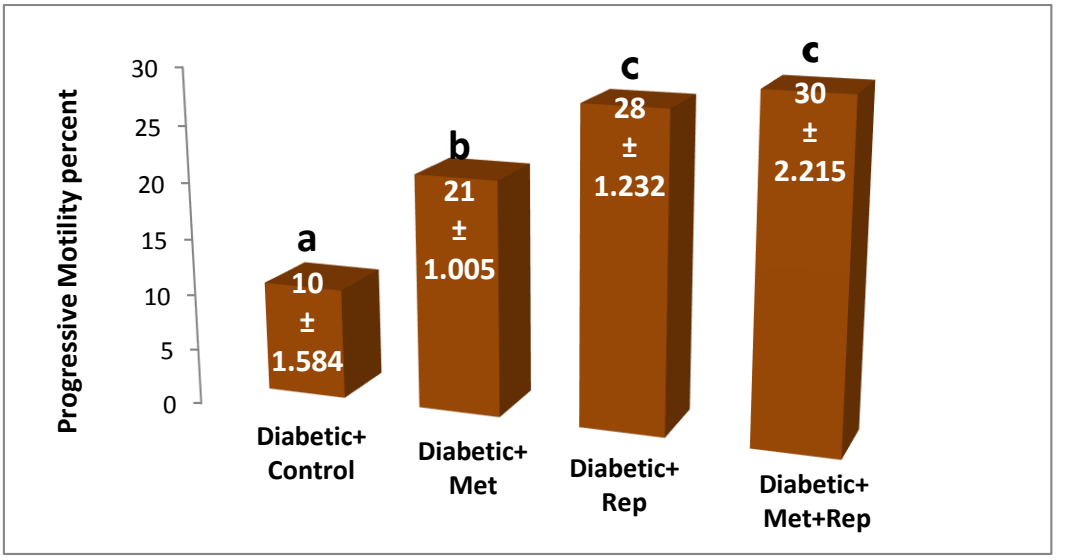

Figure 3. Sperm progressive motility percent of diabetic rats. Different letters demonstrated considerable variations at P<0.05 (mean± SE.)

These findings may indicate the direct influence of diabetes complications, which decrease sperm motility. It might also relate to the drug's enhanced impact on reducing diabetic complications. Several routes in diabetes mellitus may cause oxidative damage. Male infertility is linked to oxidative stress and reactive oxygen species produced by these processes (Mangoli *et al.*, 2013). Oxidative stress might lower testosterone levels, alter the shape of the seminiferous tubules, and result in a failure of spermatogenesis. 30% to 40% of male infertility cases had high ROS concentrations in their semen, according to research (Ribas-Maynou and Yeste, 2020). Sperm motility, viability, count, and testosterone levels have all been exhibited to be significantly reduced in animal models of DM (Nak-ung *et al.*, 2018).

Metformin could improve sperm quality by promoting the antioxidant ability of the testis (Fang *et al.*, 2012). Moreover, motility, epididymal sperm count, and morphology were enhanced in diabetic rats following treatment with 30 mg/kg per day of metformin for 6weeks (Adaremoye and Lawal, 2014). Repaglinide has a considerable positive impact on oxidative stress indicators in T2 diabetes individuals (Gumieniczek, 2005).

Treatment with repaglinide substantially enhanced total serum antioxidant capacity and serum SOD. Another study concluded oxidative changes provoked in the testis of rabbits by hyperglycemia. It reduces with repaglinide treatment at a therapeutic dose (Gumieniczek *et al.*, 2007). Guardado-Mendoza (Guardado-Mendoza et al., 2013) revealed that repaglinide is beneficial as monotherapy or in combination with metformin or other anti-diabetic medications. Its fast action duration and dosing before every meal make it an effective therapy for type 2 diabetes patients.

Figure 4 demonstrates a substantial increase ($P<0.05$) for sperm progressive motility in non-diabetic rats and the group of non-diabetic rats treated with repaglinide compared with other groups of rats treated with metformin and the combination of treatments. According to this study, metformin reduces sperm motility via lowering testosterone levels, which results in spermatogenesis problems.

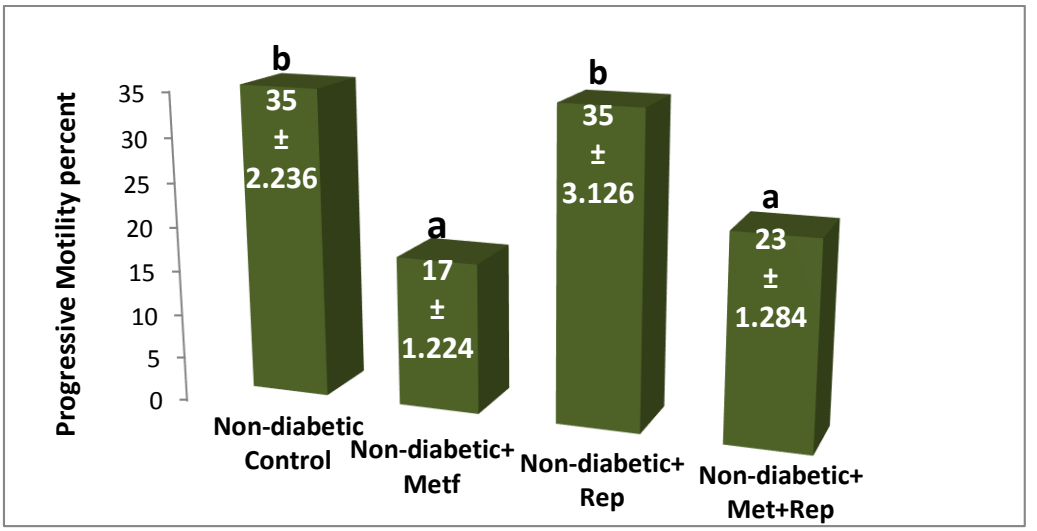

Figure 4. Sperm progressive motility percent of non-diabetic rats. Different letters demonstrated considerable variations at P<0.05 (mean± SE.)

Non-diabetic obese men who take metformin and follow a low-calorie diet exhibit lower free testosterone levels and sex hormone-binding globulin. It also reduces total testosterone (Ozata *et al.*, 2001). The influence of diabetes treatment on testosterone levels was also researched. Metformin considerably lower testosterone synthesis by inhibiting a key enzyme, Cytochrome P450-C17a, and inhibits the LH hormone release (Valsamakis et al., 2013).

Several hypoglycemia drugs have side effects (SUs, insulin, meglitinides). It has to be known how this may affect male reproductive function, given its great dependence on energy (Tavares *et al.*, 2019). The administration of drugs in the group of sulfonylureas or meglitinides showed a positive effect on the sperm and testicular levels. It reverses the effects promoted by diabetes (Nelli and Kilari, 2013) and non-diabetic rats (Adaramoye *et al.*, 2012).

It relies on stimulating the genetic code of Ca^2 channel synthesis by repaglinide during spermatogenesis. Because repaglinide control intra-sperm calcium concentration Ca^2, it is recognized to have a critical function in regulating motility and viability of ejaculated spermatozoa, resulting in depolarization of the cell membrane. It stimulates the voltage-gated calcium channels, leading to increased Ca^2 influx, finally raising the intracellular Ca^2 level (Kumar *et al.*, 2008).

Conclusions

Diabetes mellitus type 2 (DM2) is a major public health issue. It was believed that DM had no impact on male reproductive function for a long time. In this research, diabetic and non-diabetic albino rats were given repaglinide or metformin to regulate sperm motility and testosterone levels. The conclusions of the current study are:

- The best dose of alloxan to induce T2DM is 120 mg/kg in triple doses.

- Repaglinide can increase body weight by increasing glucose consumption and storage compared to Metformin, decreasing body weight. Diabetic treatment can improve sperm progressive motility percent and prosperity sexual activity.
- Repaglinide enhances testosterone levels for diabetic animals.
- In diabetic rats, a combination of Metformin and Repaglinide medications improves sperm motility and restores testosterone levels.

The current study paves the way for future investigations, including:

- Evaluating the impact of medications on the same parameters for humans.
- Performing a genetic study for repaglinide effects on CatSper-1 protein depends on this drug's stimulation ability to increase CatSper protein expression.
- Investigating the impact of repaglinide on sperm parameters in vitro.
- Examining the impact of repaglinide on oxidative stress reduction.
- Histologically examining the testes and epidydimal tissues to determine the impact of repaglinide.

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