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Effect of Repaglinide and Metformin As Anti-Diabetic Drugs on Epidydimal Sperm Parameters

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Abstract

Background: Diabetes Mellitus type 2 comprises 90 - 95% of the total cases of diabetes worldwide, Because of the pandemic rise of diabetes and its high incidence among young boys, this is a critical concern, as it is critical 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 albino rats sperm parameters**(2) Methods**: The hyperglycemia was induced by alloxan in male rat model after injection of alloxan 3 doses of 120 mg/kg intra-peritoneal was used for the induction of diabetes mellitus type 2. 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) 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 and each of them contain 7 rats, also classified into same first group and treated by same doses of treatments. After 50 days of gavaging and sacrificing the animals for test epidydimal sperms parameters. **(3) Results** revealed significant decrease ($P<0.05$) in control diabetic group compared to non-diabetic in the levels of epidydimal sperm concentration, significant reduction ($P<0.05$) in abnormal sperm morphology percent of diabetic group compare with non-diabetic all treated by mixture of drugs, and significant elevation ($P< 0.05$) of progressive motility percent in the group diabetic rats compared to non-diabetic rats which treated by mixture of drugs**. (4) conclusion:**Mixture of treatments metformin and repaglinide have very positive effect on diabetic animals and enhancement sperm parameters, but metformin increase abnormal morphology percent of sperms for non-diabetic rats and increase non-motile percent of sperm for diabetic rats.

Keywords: diabetes, repaglinide, metformine, sperm parameters, epidydimal, fertility.

Introduction

All parts of the male reproductive system make as target for diabetes damaging effects. (Tavares *et al*., 2019). Thedeteriorating disease was described to influence, not only on sperm quality and function (Mangoli*et al*., 2013), but also affected on hypothalamus pituitary gland axis (HPGA) (Schoeller*et al*., 2012), testicular function

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and sperm production (Tavares *et al*., 2016), epididymis (prostate and seminal vesicles) (La Vignera*et al*., 2012), and ejaculatory function and erectile (Penson*et al*., 2009). Because of the rising diabetes epidemic and the high frequency of diabetes, this constitutes make as a crucial area for research, and it is important to assurance the reproductive health of diabetic patients during anti-diabetic treatments (Tavares *et al*., 2018).

Metformindecreases hepatic glucose creation, stimulate glucose uptake and risesensitivity for insulin in peripheral tissues without producing hypoglycemia (Rena *et al*., 2017). It has displayed to make the slightest histological changes on the structure of the testes, and help to restore testosterone levels, when compare with other diabetic treatments (Song *et al*. 2016). demonstrated undesirable effects of metformin at the level of human reproduction, Calle-Guisado*et al*., (2019) reducing sperm motility and inhibiting signalling pathways essential for the correct functioning of these cells.

Repaglinide possesses the same role of sulfonylureas, it also bind with the sulfonylurea receptor in β-cells of the pancreas and stimulate insulin secretion (Guardado-Mendoza, 2013). However, it is weaker than sulfonylurea in binding with the receptor, and thus reflected the small time of action in insulin secretion, which gives ability for any time of administration (PakkirMaideen*et al*., 2018).Besides, other results were achieved with the recognized of K-ATP channels in epididymal epithelial cells from several mammalian species (Tavares *et al*., 2018), some researchers recommended that they may be involved in numerousmannershappening along the epididymal epithelium, such as protein excretion, fluid-electrolyte transport andvital for sperm maturing (Breton *et al*. 2019). Meanwhile the occurrence of K-ATP channels on spermatogenic cells has been recognized, and have important role in sperm capacitation and its associatedactions, it is essential to analyze potentialproducts of drugs oral uptake in diabetic patients (Tavares *et al*., 2019). Repaglinideused as monotherapy or in mixture with metformin or other anti-diabetic treatment, it has small time of action and administration before to each meal take it(Guardado-Mendoza, 2013).

This study was aimed to investigation about effect of repaglinide and metformin as anti-diabetic drugs on rats fertility by evaluate the epidydimal sperm parameters.

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 (*Rattusrattus*) 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

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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)

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)

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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.

The Epididymal sperm parameters

Sperm concentration

The left epididymis was weighed after it was removed. It was cut to small piece by a sharp scalpel to release the sperm, after it was placed in 1 ml of the physiological fluid (5% Glucose solution). The solution, then mixed thoroughly and a drop was taken on a clean glass slide and examined by a microscope under power 40X. The number of sperm was counted in 10 fields and the umber was multiplied by 10^6 to known the sperm concentration in 1 ml of epididymis (Hinting, 1989).

The Percent of Sperm Motility

A drop of the epididymis solution was taken and placed directly on the slide to calculate the percentage of the motile sperm in 10 random fields and for each class according to the following equation :

$$\text{The percentage of sperm motility} = \frac{\text{Number of motlile sperms}}{\text{Total umber of sperms}} \times 100$$

The sperm motility was calculated according to (WHO, 2010) to the three major classes:

- Sperm with progressive motility
- Sperm with non-progressive motility
- Immotile sperms.

The Percent of sperm Viability

From the same solution was prepared from epididymis, a drop was taken and placed on glass slide. Add a drop of eosin-nigrosin and mixed two drops gently by another slide and left to dry, then examined by the microscope under the power 40X. Two hundred sperm were calculated to find the percent of sperm viability based on pigmentation of them or not, then viability detected by the following equation:

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(Zeneveld and polakski, 1979).

The Percent of Morphologically Abnormal sperms

The same slide used to study sperm viability was used to study abnormal sperm through the study of head, mid piece and tail abnormalities, the location of cytoplasmic droplet and the mid-piece abnormalities. The abnormality of sperms was calculated under power 40X. Two hundred of sperms were counted, the abnormality of sperm calculated according to the following equation :

Results and Discussion

Epididymal sperm parameters

Sperm Concentration

The results shown significant reduction ($P<0.05$) of epididymal sperm concentration in diabetic rats compared to non-diabetic rats, which may be refer to the direct effect of diabetic complications on rats sperm concentration; Diabetes impact on male fertility by several mechanisms such as defective spermatogenesis or losses in sperm parameters such as count motility of sperms, other factors of lifestyle have had a importanteffect on health and the capability to produce and growth of diabetes whichamplifieddanger for infertility (Aboua*et al*.,2013). Other studies on streptozotocin (STZ) in animal (Rats) model for diabetes suggested that DM caused hormonal disorder, that caused reduction in sperm counts, motility, and acid phosphatase production of prostatic,alsoincrement the fructose levels of seminal, the treatment by insulin of diabetic rats improved counts and motility of sperm, and helpful in restore the seminal fructose to control levels (La Vignera*et al*, 2012).

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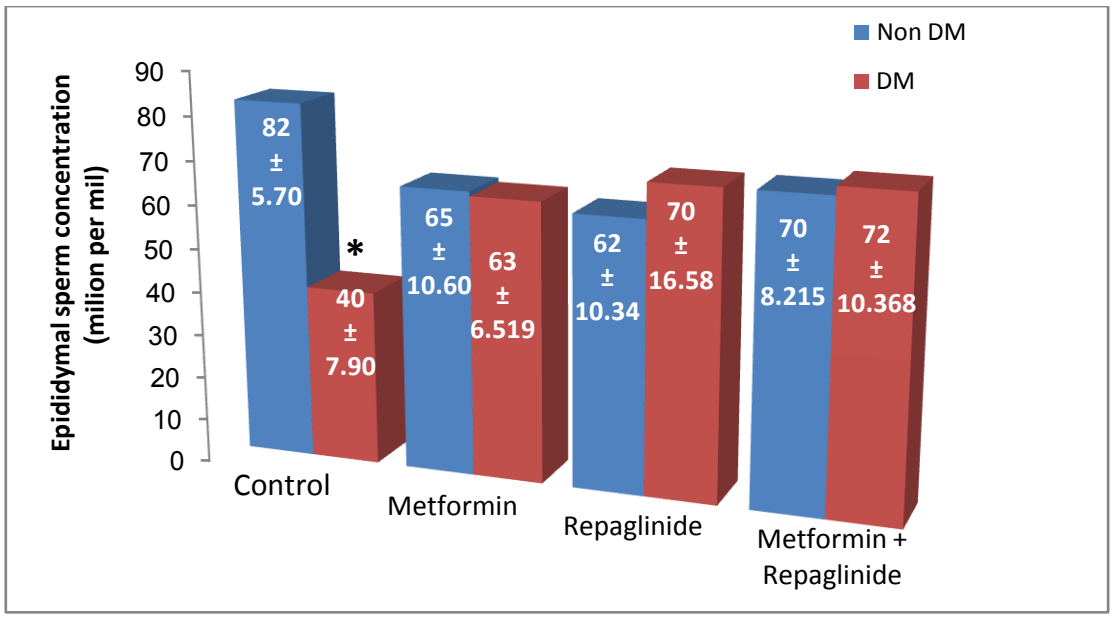

Figure-1 Epididymal sperm concentration levels significant differences at (P<0.05) (mean± S.D.)

Our results showed no significant differences (P>0.05) between groups treated by metformin diabetic and non-diabetic animals, may be refer to the enhancement effect of drug that decrease significant differences between diabetic and non-diabetic rats in epididymal sperm concentration; Adaramoye and Lawal, (2014) revealed the motility of epididymal sperm were enhanced in rats have diabetesafter metformin treatment with 30 mg/kg per day for six weeks, also the obesity rats (after diet induced) administered metformin orallyfor 12 weeks cause increased thesperm count and motility extracted from epididymus (Fang *et al.*, 2012). Additionally this treatment has been described to restore sperm feature by helping and increase motility and normal morphology of sperm, and overall, reducing sperm genomic variability caused by fragmentation ofDNA in diabetic rats (Attia*et al*. 2009). Metformin may increase the reproductive activity of male diabetic patients by inhibition cell apoptosis in germ line, such outcome may becausemetformin ability to decreaselipid peroxidation and oxidative damage also enhance AMPK activity(Alves*et al*., 2014), which lead to repair the production ofpituitary gland hormones and restore the normal levels (Banihani, 2016).

Also results revealed no significant differences (P>0.05) between diabetic rats and without diabetes inepididymal sperm concentration after administrated by repaglinide, which may be refer to the improvement effect of drug and decrease diabetes complications, repaglinide helpinsulin secretion by inhibition K-ATP channels, growing intracellular calcium concentration (Malaisse, 1995), and bind with the same receptor, the sulfonylurea receptor (SUR) (Dabrowski*et al*. 2001), that may propossed to be superior to sulfonylurea receptor, and don't cause hypoglycemia (Landgraf, 2000)

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Repaglinide make as important stimulator for insulin secretion, and it have important role in the cellular activity (Takahashia*et al*., 2018), Another study found that insulin improves diabetic male reproductive health through restoring the hypothalamus pituitary gland axis, and hence testosterone and LH levels, also having a direct interaction with the testis and sperm generation. (Schoeller*et al*., 2012).

Figure-1 revealed no significant differences (P> 0.05) between diabetic and non-diabetic rats gavaging by mixture of drugs (metformin and repaglinide) in the concentration of epididymal sperm, may be refer to the treated of diabetic complications which cause reduction in sperm concentration and these drugs don't have impact on non-diabetic rats, Bhasin*et al*., (2007) showed infertility occur by low sperm count, which due to testes production less than normal level; And La Vignera*et al*., (2009) revealed fertility in diabetic animal models andmen, is reduced, while metformin is veryeffective with their enough production of insulin (Chaudhury*et al*., 2017), as repaglinide responsible for increase and enhancement insulin secretion (Tahrani*et al*., 2016), that may be lead to decrease DM complications and development of diabetic rats fertility.

The liver is the centralsite of metformin action, also it can work in other tissues and organs, such as parts of reproductive system (Tavares *et al*., 2018).Others researchers were reportedrise in serum testosterone levels by metforminand an improvement of diabetic patients (Song *et al*. 2015); Metformin can modulate and improve pituitary LH and regulate Leydig cell steroidogenesis in testes (Tavares *et al*., 2018). The serum levels of prolactin, luteinizing hormone, follicle stimulating hormone and testosterone were describedlike to the untreated diabetic males and reduced when comparison to the non-diabetic animals(Adaramoye and Lawal, 2014). These treatments make together for endocrine balance and helpful to reproduction ability, numerous research reported the correlation between diabetes and sexual hormones, also other studies refer to the effect of diabetes treatment on sexual efficiency (Tavares *et al*., 2018); Repaglinide is important agent to insulin secretion during glucose entrainment in Type 2 diabetic patients (Guardado-Mendoza, 2013), suggesting a important function for insulin in the reproductive tract and an increased potential for diabetes to affect male fertility (Tavares *et al*., 2018), insulin was revealed to regulation LH and FSH release in pituitary cell cultures (Adashi*et al*. 1981) and interaction with the hypothalamic pituitary gland axis, also refer to gonadotropin releasing hormone (GnRH) secretion(Burcelin*et al*. 2003).

Abnormal Sperm Morphology Percent

The result revealed significant elevation (P<0.05) in abnormal sperm morphology percent of diabetic rats compared to non-diabetic rats, which may be refer to the direct effect of diabetic complications on sperm morphology in diabetic animals; Nak-ung*et al*., (2019) reported the alterations of sperm morphology include:nucleus, acrosome, plasma membrane and mitochondria in diabetic patients, also significant diminutions in testosterone levels, and viability, motility and count of sperms; The patients of

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types 2 diabetes mellitus have low volume, abnormal motility and morphology compared to non-diabetic subjects (Ibrahim *et a*l., 2018). Other study showed a main factor is oxidative stress which in diabetes mellitus, and also has detrimental effects on sperm parameters compared to non-diabetic group (Singh *et al*., 2014).

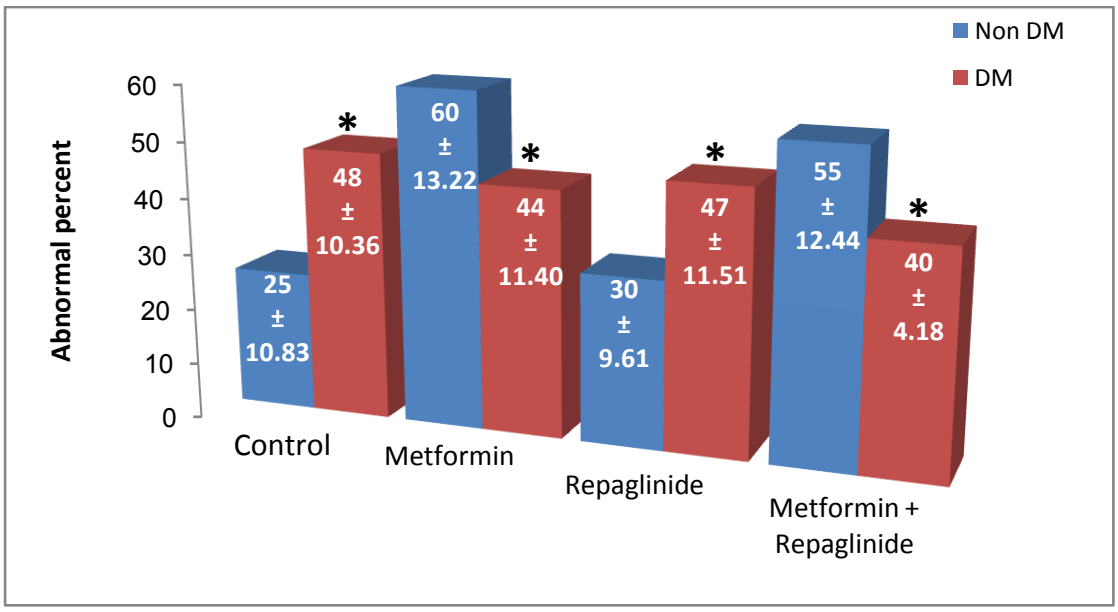

Figure-2 Abnormal sperm morphology percent significant differences at (P<0.05) (mean± S.D.)

Also may be refer to the sexual hormonal disorders which lead to spermatogenesis impaired and increase sperm abnormal morphology, sexual hormones have important actions inspermatogenesis (Zhao *et al*. 2020); Type 2 diabetes have important impact on imbalance in sex hormones (Gambineri and Pelus, 2019), Sex hormones, include testosterone, follicle-stimulating hormone,luteinizing hormone, estradiol (E2),and sex hormone-binding hormone (SHBG), are proved to play vigorous roles in sperm maturing and spermatogenesis (Patel *et al* . 2016).

The results showed significant decrease (P<0.05)theabnormal percent of sperm in diabetic rats compared to non-diabetic rats treated by metformin, which may be refer to the effect of metformin on diabetic rats lead to enhancement spermatogenesis by decrease diabetes complications, the various homeostatic and biochemical alterations of male infertility that occur by DM, patients of diabetic have designatedproblems in sexual activity such as weakened desire of sexual work, that directly related with hyperglycemic state (Ewing, 1985)

Diabetes mellitus makedeficiencies in ability of male reproductive functions, spermatogenesis is decreased in diabetic patients (Maresch*et al*., 2018). Numerous researchmake comparison between young or adult diabetics with control persons, and shown that diabetic males have poorercounts of sperm and major changes in motility and morphology of sperm (Baccetti*et al*., 2002). Metformin aids to renovate the body's response for insulin, also metformin decreases complications occur with

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highly blood glucose level (Zaidi*et al*., 2017). Also may refer to the increase morphology alteration of non-diabetic rats treated by metformin, that agreement with study was concluded thatmetformin use in association with hypocaloric diet reductionsex-hormone-binding globulin levels and free testosterone in non-diabetic obese men, and reductions total testosterone levels in obese patients with diabetes type 2 which cause hormonal disorder and spermatogenesis defect (Ozata*et al.*, 2001). But Rabbani, *et al*., (2010) revealed the oral usage of 50 mg/kg/day metformin, for four weeks in diabetic rats,mixture with pioglittazone, another anti-diabetic treatment, at 1 mg/kg decreases sperm morphology defects and increase sperm count in the caudal of epidydimus; Also, count, motility, and morphology of epididymal sperm were improved in diabetic rats upon treatment with metformin (30 mg/kg per day at for six weeks (Adaramoye and Lawal, 2014).

The significant elevation ($P<0.05$) of abnormal sperm morphology percent showed in the group of diabetic when compared with non-diabetic rats which administrated by repaglinide, that may be refer to the diabetic effect which increase abnormal morphology percent of diabetic rats, and may be repaglinide decrease hyperglycemic but without improved abnormal sperm percent, diabetes mellitus, and performed to identify variations in the testicular sections of diabetic rats with affections to count of sperm, width of the germinal epithelium andLeydig cells number, all of these portions in male reproductive tract are improved the spermatogenesis (Yelumalai*et al*., 2019). An oppositerelationship has been observed between quality of sperm and blood glucose level in men and animals (Alves*et al*., 2013). Seminalinvestigates in men ofdiabetic revealed reducedmotility of sperm (La Vignera*et al*., 2012) sperm concentration (Delfino, *et al*., 2007) but increased abnormal morphology of sperms (Fabian *et al*., 2016). Diabetes Mellitus is reported to be related with oxidative stress, a formal in which genes associated with intracellular signaling, DNA reliability, sperm structure and motility are altered (Mallidis, *et al*., 2009). Oxidative stress can also led to sperm damage caused by increase the level of reactive oxygen species (ROS), in diabetic patients, the level of reactive oxygen species can exceed antioxidant ability in seminal plasma (Singh *et al*., 2014). Alsoproducing increase the oxidative stress, and hyperglycemia may cause inflammation and apoptosis (Shahzad, *et al*., 2015).

Figure-2 showed significant reduction ($P<0.05$) in the group of diabetic compared with non-diabetic rats treated by mixture of drugs metformin and repaglinide in abnormal sperm morphology percent , which may be refer to the synergisticcurative effectof two treatments on diabetic rats, and may be improvement whole body diabetic disorder, several studies in both animals and human have definitedamaging effect of diabetes mellitus on function of sexual production, such as nuclear fragment of DNA for sperm, sperm parameters,abnormal homeostasis of glucose,and chromatin quality, all that have adverse effects for the reproductive system (Mangoli*et al*., 2013)

Spermatogenesis and testicular function make as important target for DM type 1and 2 because complications in diabetic patients (Agbaje*et al*., 2007), the normal

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examination by light microscopic of diabetic man ejaculate, thinking that the influence of diabetes is small on seminal quality, and molecular techniques in researchthatconfirmed the truth ofdramatically highly percent of sperm with mitochondrial DNA fragmentation and abnormal sperms of diabetic person (Ding, *et al*., 2015).

Fakhrlidin*et al*., (2016) concluded the percentage of abnormal sperm morphology (ASM), have highly significant increment (P<0.01) for low dose as compared with control group, while mid dose appeared highly significant decrease in the ASM (%) when compared with control and low dose groups. Meanwhile, high dose observed highly significant increase (P<0.01) in the ASM (%) when compared with control and other two treated groups, and the reasons for that may be occur by metformin causes decrease testis size, weight, testosterone production and reduction size of Sertoli cells which lead to spermatogenesis impaired and increase abnormal sperm morphology percent (Adaramoye, *et al*., 2012).

The important role of repaglinide is regulator for glucose level and used in the administration of diabetes mellitus type 2, that make in attachment withβ-cells of the pancreas to activate insulin excreation(Hollingdal *et al*., 2005). While the potential of other oral hypoglycaemictreatmentsusing for diabetes has well been examined, there is little information about support their defense against oxidative damage occur during diabetic complications, which caused hormonal disorder and spermatogenesis impaired (Obi *et al*., 2016), increment of abnormal sperm morphology of non-diabetic rats that may be refer to the opposition effect of drugs.

Sperm Viability Percent

The resultshowed significant reduction (P<0.05) in the percent of sperm viability of rats that have diabetes, compared to other is non-diabetic, may be refer to the disease effect on diabetic rats led to decrease viability percent, Zheng*et al*., (2016) showed LH and FSH levels were decreased in the low testosterone groups, such that testosterone was positively correlated with LH and FSH. The reduction of these hormones may be resulted from the diabetes oxidative damage to the hypothalamus which responsible for the secretion of gonadotrophin-releasing hormone (GnRH) that stimulates LH and FSH release by the pituitary gland which in turn inhibit testosterone biosynthesis in the testes of rat (Umosen and Chidiebere 2014). These hormones testosterone, FSH and LH plays a crucial role in initiation, maintenance and maturation of the Spermatogonia (Oduwole, *et al*., 2018).

Also the results showed significant reduction (P<0.05) of sperm viability percent in diabetic animal and compared with non-diabetic rats all treated by metformin, that may be refer to the diabetic effect on rats which decrease viability percent also after treated by metformin, and may be this drug have positive effect by increase viability percent in non- diabetic rats, diabetes mellitus led to histologic destruction in the epididymal tissues, with a adverseeffect on spermatogenesis (La Vignera*et al*., 2012).

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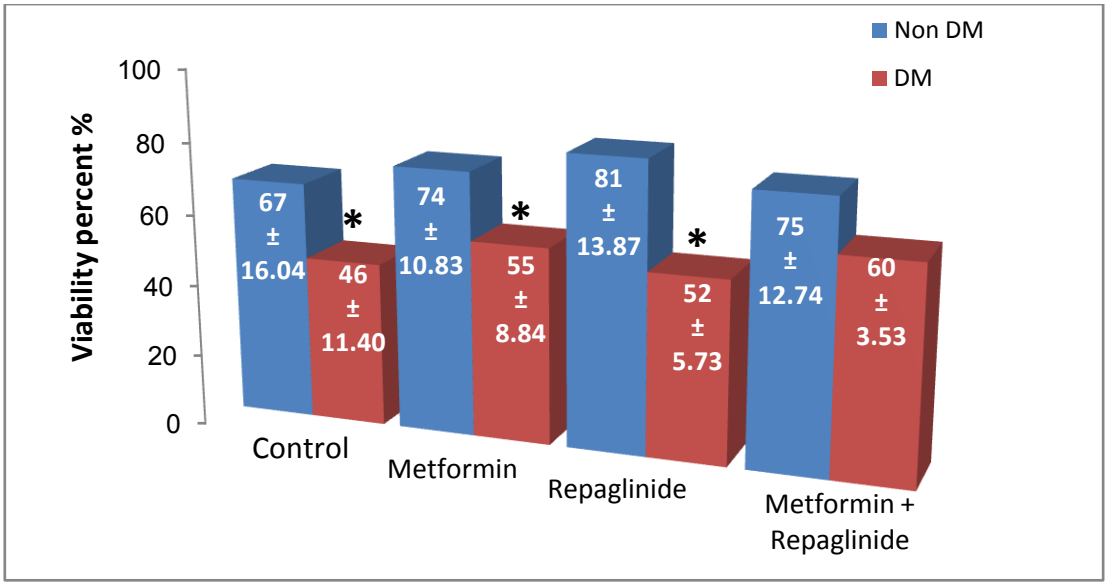

Figure-3 sperm viability percent significant differences at (P<0.05) (mean± S.D.)

Several ways may clarify the damage of sperm detected in diabetes patients, these comprisecomplaints of endocrine, increased oxidative stress and neuropathy, (La Vignera, *et al*., 2009). Oneapparent mechanism is that metformin decreases the level of damage occur by oxidative stress (Banihani*et al.*, 2014), depend on usedmetformin in 30 mg/kg supplementation at repairs the antioxidant function (Adaramoye, Lawal, 2014). Resembling findings of improving the antioxidant function were detected by diet-induced obesity rats. such as higher superoxide dismutase and glutathione peroxidase, and lower malondialdehyde (Fang *et al.*, 2012).Metformin was recognized as taking antioxidant activity against diabetic oxidative destruction by reactive oxygen species (ROS) (Cahova*et al.*, 2015).

Figure-3 revealed the significant reduction (P<0.05) of viability percent in diabetic compare with non-diabetic rats administrated by repaglinide, which may be refer to the diabetic effect on rats, caused spermatogenesis impairment which led to decrease viability percent also with treated by repaglinide, but in non-diabetic rats the treatment may be enhancement viability percent.

Clinical studies showed diabetes mellitus is associated with poor sperm parameters, diabetes have ability to influence in the expression of genes in DNA of sperm, causing in a greatdegree of nuclear DNA fragmentation anddeletions DNA of mitochondria (Roessner, *et al*., 2012). It has been exposed that acrosome and sperm plasma membrane are highly affected by serum insulin levels, so, through insulin resistance or insulin deficiency, spermatogenesis is changed and biopsies of diabetic men showed testicular variations (Condorelli, *et al*., 2018). Also diabetic patients develop hypogonadism and alterations of hypothalamic GnRH (Pitteloud, *et al*., 2005), or peripheral variation infunction of Leydig cell, the mechanisms cause decrease in serum gonadotropin and testosterone levels (Condorelli, *et al*., 2018). Furthermore, there have been information on alterations in morphology of sperm

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including the plasma membrane, nucleus, acrosome, and mitochondria in patients of DM, similarly occur indiabetic animal models, cause significant decreases in viability, count, and motility of sperm testosterone levels that was shown,. (Nak-ung*et al*., 2019).

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 function of male reproductive system is well reflected bymany researches, insulin action is effect on all portions of male reproductive system and hypothalamus pituitary gland axis, likewisetaking importantrole in function erectile and semen ejaculation, also in male accessory organs, insulin was shown to interfere with the hypothalamic pituitary gland axis, and stimulating release of LH and FSH from pituitary cell culture(Adashi*et al*., 1981), and secretion gonadotropin releasing hormone, also in expression in hypothalamic neurons in culture (Burceli*net al*., 2003).

The alterations occur during diabetes mellitus, resemble decrease in progeny, spermatogenesis impaired, in epididymal sperm degeneration,reduction in leydig cells population and decrease serum LH concentration (Bruning*et al*., 2000). The enhancement of insulin levels might also act directly on receptors of insulin as well as insulin expression has been described in the testes, pointing to an important role of insulin in regulation the spermatogenesis by provide energy for cell important processes (Gomez*et al*., 2009).

The results revealed no significant differences (P>0.05) between diabetic and non-diabetic animals which treated by mixture of drugs (metformin and repaglinide), may be refer to the effect of disease on diabetic rats, also may be refer to the direct improvement effect of two drugs on non-diabetic rats which decrease diabetic complications.

Some studies showed that intensified oxidative stress which is following elevated blood glucose implicated in creating diabetes complications, lowering serum concentration of testosterone besides the negative impact on the reproductive system like reduction in accessory sex glands weight, reducing sperm content in the epididymis and increasing basement membrane thickness is reported in diabetic patients, all of that caused spermatogenesis defect (Nasrolahi, *et al*., 2013).

Metformin is a hydrophilic biguanide compound with high polarity, positively charged, and has low molecular weight with pleiotropic actions. It extensively accumulates in some tissues including the liver, pancreas, muscle, adipose tissue, pituitary, hypothalamus, and the gonads (Bertoldo, *et al*., 2014); A study provide evidence that metformin improves sperm motility, count, concentration, and antioxidant status and decreases oxidative stress (Alzain, *et al*., 2020).

Other study showed the strong protectingrole of metformin on testes and injury restorationin rats, DM decrease actions of superoxide dismutase (SOD) enzyme, organized with an rise in myeloperoxidase (MPO), as well as malondialdehyde

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(MDA) levels, metformin had restored testicular Johnsen's scores, SOD activity, regulation MPO and MDA levels (Asghari, *et al*., 2016).

Repaglinide regulated activity of glutathione disulfide reductase(GSSG-R)in testes cell, as well as the levels of glutathione (GSH) and ascorbic acid, at the same time significantly decreased production of lipid peroxidation, that cause there was an initial increase in endogenous antioxidant activity due to excessive production of reactive oxygen species (ROS) (Gumieniczek, 2005). At the same time the antioxidant protection was inadequate to remove extreme free radicals, resultant in the incidence of oxidative stress, repaglinidedecrease oxidative stress with by enhanced anti-oxidative defense and reduction in lipid peroxidation (Assaloni*et al*., 2005).The level of ascorbic acid increasing in diabetic tissue after repaglinide was parallel to that saw in the plasma and heart cell of rabbits that diabetic was induced, thedecline of lipid peroxidation level in testes cell of diabetic men after repaglinide was similar to its effect detected in the serum of type 2 diabetic patients (Gumieniczek, *et al*., 2007). Repaglinide havepotential role by exert some antioxidative properties through breaking the chain reactions or reducing the oxidation rate (Natella, *et al*., 1999).

Sperm Motility

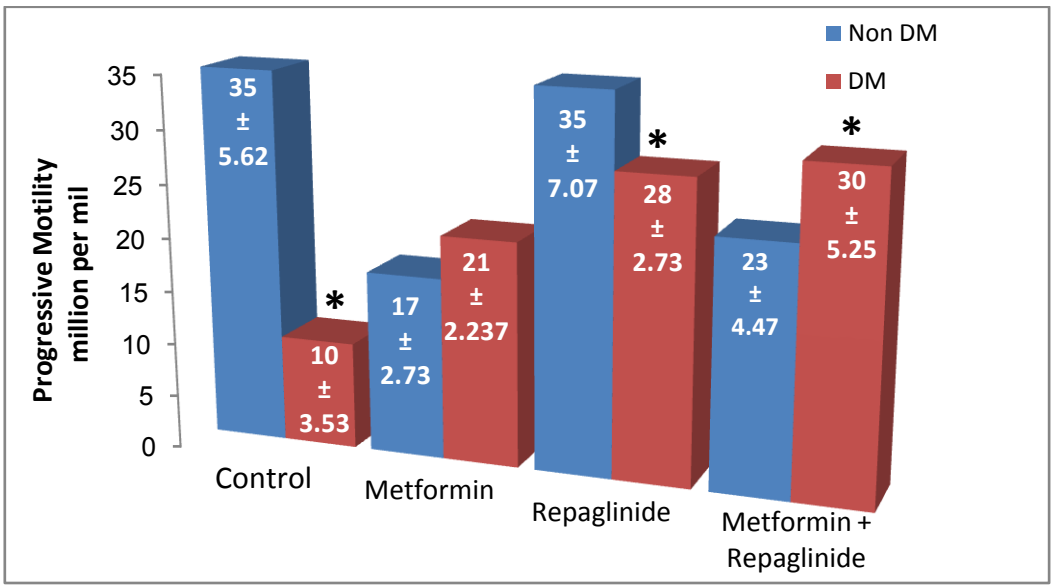

Figure-4 Progressive Motility percent
significant differences at (P<0.05) (mean± S.D.)

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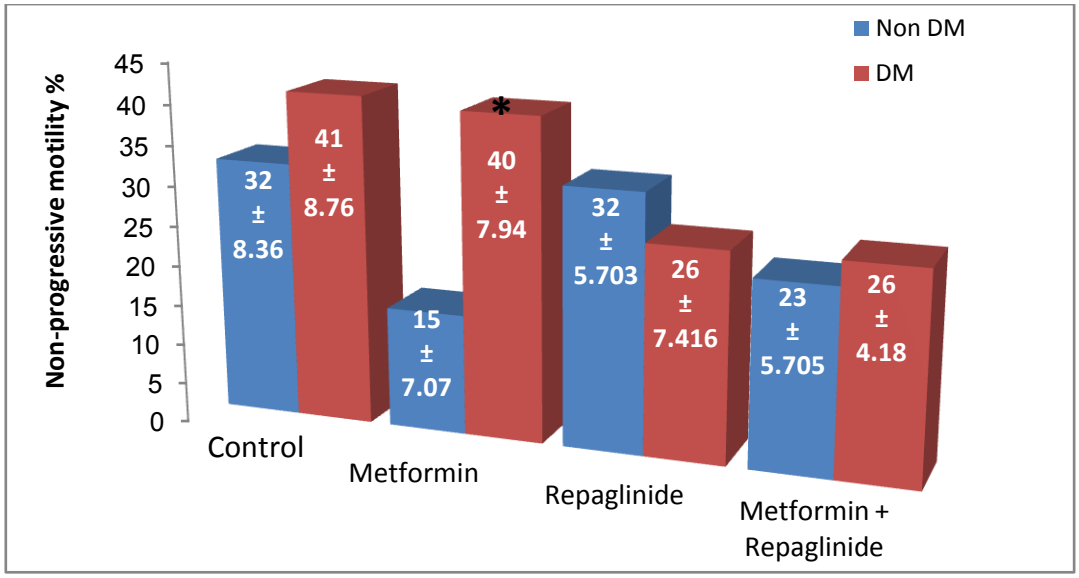

Figure-5 non-progressive motility percent significant differences at (P<0.05) (mean± S.D.)

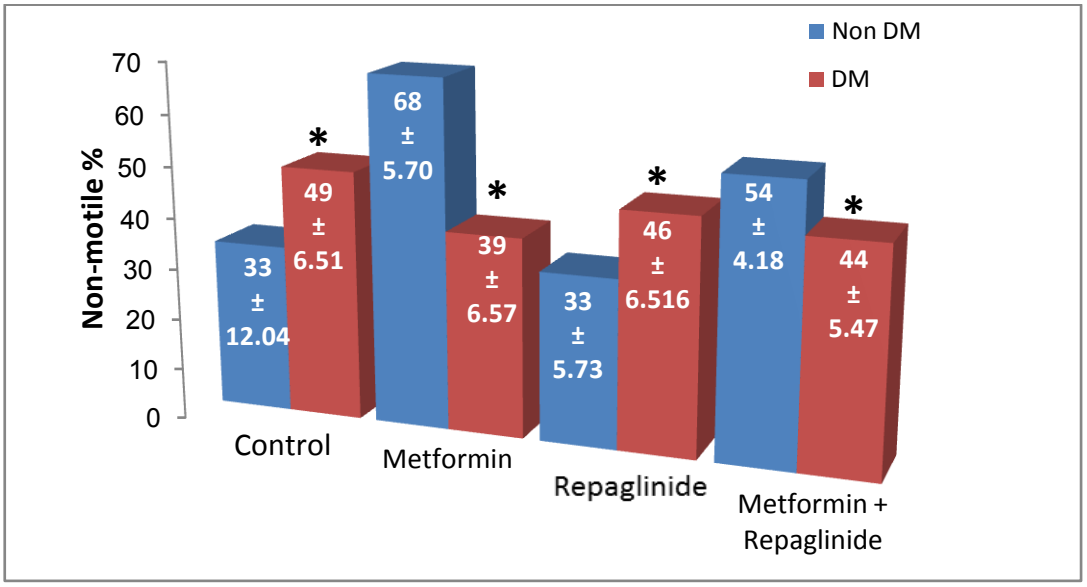

Figure-6 non-motile sperm percent significant differences at (P<0.05) (mean± S.D.)

The results showed significant decrease (P<0.05) of progressive motility percent in diabetic rats compared non-diabetic rats (figure-4), and significant increase (P<0.05) of non-motile sperm percent in group of diabetic rat compared to non-diabetic rats (figure-6), but results revealed no significant differences (P>0.05) between diabetic control group and non-diabetic rats in non-progressive motility percent (figure-5).

May be refer to the effect of DM which caused decrease progressive motility percent and increase non-motile percent in diabetic animals, the evidences of diabetes has opposing effect, that causechanges in testes structure, seminiferous tubules and decrease sperm quality, as well as deleterious in spermatogenesis, these changes may due to high production of ROS as a result of hyperglycemia in the rat model of

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diabetes, leading to impaired function of all structures complicated in production process of sperms and development (Nak-ung*et al*., 2019). Other study revealed diabetes mellitus caused reduced semen ejaculation and reducedspermatozoa motility and vitality (Miralles-Garcia and Garcia-Diez, 2004). Many systemic complications caused by diabetes include,hypogonadism, retrograde ejaculation, male infertility, and impotence, (Dohle, *et al.*, 2010). Resistance of insulin is an important threat factor for T2DM, defects in insulin secretion may change testicular and accessory sex glands function, (Bener, *et al*., 2009). Moreover, glucose metabolism is important for maintaining basic cell activity, as well as specific functions, such as motility and fertilization ability in mature sperm (Ding, *et al*., 2015).

Figure-4 showed no significant differences (P>0.05) in progressive motility percent between diabetic and non-diabetic rats treated by metformin, but notice significant elevation (P<0.05) of non-progressive motility percent in diabetic rats compared with non-diabetic rats after metformin administrated (figure-5), and significant reduction (P<0.05) of non-motile percent for diabetic compared with non-diabetic rats after treated by metformin (figure-6), may be refer to the effect of metformin on diabetic rats which removal differences between diabetic and non-diabetic rats in progressive motility percent and increase non-progressive percent in diabetic rats, but may be have negative effect on non-diabetic rats by increase non-motile percent.

Metformin decreases free testosterone and sex-hormone-binding globulin levels, when using in combination with a hypocaloric diet (Ozata*et al.,* 2001). The effect of diabetic pharmacotherapy on testosterone levels, metformin significantly reduces testosterone; through inhibition of Cytochrome P450-C17a which is a key enzyme in testosterone synthesis and reduction of LH hormone secretion (Valsamakis*et al*., 2013). Faure *et al*., (2018) refer to the positive effect of metformin and mention the capacity of it to enhance quality of sperm, and ameliorated impairment of testicular tissue, also epididymal sperm concentration and motility bydecrease germ cell apoptosis and reduction the oxidative stress.

Figure-4 revealed significant reduction (P<0.05) of progressive motility percent in diabetic rats compared to non-diabetic treated by repaglinide, that revealed significant elevation (P<0.05) of non-motile percent in group of diabetic when compare with non-diabetic rats after treated by same drug (figure-6), but results showed no significant differences (P>0.05) between diabetic and non-diabetic rats in the percent of non-progressive motility after treated by repaglinide (figure-5), may be refer to the direct effect of diabetes caused spermatogenesis defect led to decrease progressive percent and increase non-motile percent despite of treated with repaglinide, diabetes may affect male reproductive function at multiple types as a result of its effects on the endocrine regulation of spermatogenesis, sperm activity, semen quality and impairing penile erection (Jangir and Jain, *et al*., 2014).

Another study revealed a decrease in all parameters of semen which had been observed in the diabetic patient group lower than non-diabetics (Mangoli*et al.,* 2013).

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Diabetes effect on male reproductive system by insulin resistance or insufficiency, and rise of oxidative stress,resistance of insulin directly affect testis development, spermatogenesis, and the secretion of hormone related to fertility of male, due to the decrease the amount of glucose moving into sertoli cell (Abu Bakar*et al* ., 2020).

Repaglinide may cause permeability changes of sperm membrane by stimulate gene code for calcium channel building during spermatogenesis, other researcher also used repaglinide which concluded the decreaseviability of sperm in human, eventually, in the complete loss male gametes viability. This result would also suggestrepaglinide make as a spermicidal by more increase calcium ions influx that have negative effect on spermatozoa and that depend on dosage, period and mode (in vitro or in vivo) of exposure for repaglinide (Kumar *et al*. 2008).

Figure-4 revealed significant increase ($P<0.05$) of progressive motility percent in diabetic rats compared to non-diabetic rats treated by mixture of drugs (metformin and repaglinide), but no significant differences ($P>0.05$) between diabetic and non-diabetic rats, in percent of non-progressive motility (figure-5), and result revealed significant decrease ($P<0.05$) in diabetic rats compare to non-diabetic rats in non-motile sperm percent, after treated by mixture of drugs (metformin and repaglinide), may be refer to the direct effect of drugs that reduction diabetic complications which increase progressive motility percent and decrease non-motile percent for diabetic rats.

Metformin, glibenclamide, and repaglinideadministration displayeddecrease in the malondialdehyde concentration and greatenhancement in the different activities of antioxidant enzymes. This founds that they deliver antioxidant defense as anti-diabetic agents, thusprotective the pancreas from oxidative stress-induced impairment during diabetic complications (Obi *et al*., 2016). The positive effect of metformin and mention the capability of it to increasequality of sperm, and administration repair testicular tissue damage and increased concentration of epididymal sperm and motility through decrease in oxidative stress (Faure *et al*., 2018). New study 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 (Younas*et al*., 2021). Meglitinides are usually used asmixture with metformin, insulin or a thiazolidinedione, although they can be used as monotherapy (Bianchi *et al*., 2018).

Repaglinide make as important agent for stimulate insulin secretion, also called short-acting factor,with biliary excretion and rapidly stimulates insulin secretion shortly after a meal and corrects abnormal patterns of insulin secretion and decrease blood glucose level (Scott, 2012).Few studies about meglitindes, besides sulfonylureas, which determined the reductionviability of human sperm after these treatment using, which led to complete loss of all male gametes viability. However, the limited information about repaglinide, that need of further researchs (Tavares *et al*., 2018).

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Conclusions

1-There is significant differences between diabetic and non-diabetic rats in sperm parameters.

2-Diabetic treatment have ability to improvement sperm motility, morphology and concentration.

3-Metformin increase abnormal morphology percent of sperms for non-diabetic rats.

4-Metformin increase non-motile percent of sperm for diabetic rats.

5-Repaglinide enhancement sperm parameters.

6-Repaglinide increase sperm progressive motility percent.

7- Mixture of treatments metformin and repaglinide have very positive effect on diabetic animals and enhancement sperm parameters.

Recommendations

1- Estimate the effects of drugs on the same parameters for human.

2- Study the effect of Repaglinide on semen parameters in-vitro.

3- Study the reduction effect of Repaglinide on oxidative stress.

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