Effects of Metformin on Sperm Parameters in Mice: as a model for human being

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Abstract

Background:

Metformin is anti-hyperglycemic effect due to decrease of hepatic glucose production. Another effect, leading decrease insulin resistance, regarded a safe drug, treatment of choice for overweight.

Objective:

To investigate the effects of 3 concentration of MF administered orally to male mice on sperm parameters as model for human being.

Materials and Methods:

In current study, 80 male mice were taken with age ranging between 1.5-2 month and weights ranging between 25-30 grams males classified into four groups of a control group (G1) and treated group (G2, G3 and G4) doses of MF were employed, 0.2, 0.4, 0.6 mg/mice daily to groups, given orally for 6 weeks and then the animals were scarified.

Results

Results have revealed a significant difference ($P < 0.05$) in sperm concentration for 3 groups compared to G1 group. Progressive motility (%) appeared with significant difference ($P< 0.05$) for G3 group as compared to other treated and G1 group. Furthermore, the result illustrated a significant decreased ($P< 0.05$) in the abnormal sperm morphology for G3 group compared with treated and control. However, sperm agglutination (%) confirmed that the G3 group exhibited highly statistical significant decrease ($p\leq0.001$) compared to G1, G2 and G4.

Conclusions

From results of the present study, it was concluded that the administration of MF doses reduce sperm concentration .while, 0.4mg dose improve certain sperm parameters.

Key words: **Metformin,Semen analysis,Sperm parameters.**

Introduction:

Metformin (MF) is the first-line medication treatment for T2D. Globally, more than100 million patients are prescribed this medication annually [(1)]. Metformin, is the generality prescribed insulin sensitizer in the curative management of T2D [(2)].The biguanide chemical properties of MF, the drug is differentiate by a unique distribution action; following oral administration to intra duodenal or, part of the MF is reversibly intensify to the luminal surface of the intestinal wall [(3)].

The patients with T2D, MF has been associated with lowering cardiovascular (CV) mortality and morbidity, by developer in glycaemia control [(4)]. A possible decrease in cancer incidence, which was seen in some cases [(5)] [(6)] . Action of MF is its interference in oocyte maturation [(7)] .Many research have suggested actions of MF in granulosa cell steroidogenesis and oocyte maturation [(8)].

MF therapeutic action on metabolic and weight parameters [(9)]. Adipose tissue is not essential site of MF effects; however, MF appears to have modest action on this tissue. An *in vitro* search has examined how MF affects metabolic pathways in preadipocytes. Overall, MF was shown to increase catabolism, as reflected by high the glucose transport and utilization [(10)]. In human which adult contain two testis organs that lie inside the scrotum, a sac of skin amidst the upper thighs, suspended by the spermatic cord and have dual action include produce the spermatozoa and hormones [(11)]. The right testis mostly being 10% heavier than the left, mean weight of each testis is 15 to 19 g. The testis blood supplied is derived primarily from the internal spermatic (testicular) artery with a smaller contribution from the branches of the vassal portion of the internal (superior) vesicle artery [(12)].

This blood supply regular the temperature of testis by countercurrent heat exchange with the veins of the pumping form plexus ,and the heat lost via the thin scrotal covering layers serve for keep the testicular temperature 2 ºC beneath body temperature [(13)].

Materials and Methods:

The mature males mice are with an average body weight of 25-30g, the males are kept in plastic cages with a metal mice network cover under climate controlled conditions of the animal house with (22-25ºC) temperature room. Male mice were provided with water and food ad libitum. The air of the room was changed continuously using ventilating fan. Photoperiod was automatically controlled of 13±2 hrs light from 6 A.M. to 7 P.M. daily regime. The best cage for mice is an aquarium with a wire mesh measuring (29×15×12) cm. Four mice were kept in each cage, containing wooden shave. Tap water and diet were available for the animals. Male mice were provided with water and food ad libitum a mouse normally eats 4 to 5 g/day of a completed pelleted diet and drink about 4 to 5 ml/day [14].

❖ Preparation of metformin:

Metformin solution was prepared by dissolving completely a crushed one tablet (500 mg) in 8.33ml of normal saline(n.s) to prepare 1ml concentration of 6 mg of metformin this considered as stock solution ,then obtained different doses of metformin (0.2, 0.4, and 0.6) mg /male mice. To prepare doses 0.6 take 0.1ml from stock solution mixing with 0.9ml n.s high doses (0.1 concentration 0.6mg), mid dose were prepared by taking 0.1 from stock solution mixing with 1.4 n.s all 0.1 have concentration 0.4 mg, low dose we take stock solution 0.1ml mixing with 2.9 n.s to produce 0.1 ml concentration 0.2mg. Each doses administered for limited group of the males mice. In this study, the male mice were divided in to 4 groups as control and 3 treated group.

❖ Doses and period:

In this study uses metformin 0.2 equal 500 mg, 0.4 equal 1000 mg ,0.6 equal 1500 mg ,in human being

giving for male mice through 6 week without exception any days .

❖ Route of administration:

Metformin drug by orally administration once daily using modified Insulin syringe or gastric tube (1mL).

❖ **Sperm Collection**:

The sperms were collected from the caudal epididymis of Male mice according to using the following steps:

1. Male mice were sacrificed by cervical dislocation and dissected directly.
2. The caudal of epididymis of each side were isolated and placed in Petri dish containing 1 ml of SMART medium at 37°C to prevent cold shocks and minced by using microsurgical scissor and forceps[15] .Taking both left and right caudal considered sites of acquired capacity for sperm cell, and washed with normal saline then they were put in a small well of Petri-dish contained 1ml SMART media then cauda of epididymis were minced by microsurgical scissor about 200 times until getting a homogenized solution [16].

❖ **Assessment of mouse spermatozoa:**

One drop taken about 10µl of spermatozoa suspension was mounted placed on a warm microscopically class slide and covered with a cover slip (22x 22 mm), then left for one minute in an incubator to stand before microscopic examination' Calculations were as assessed as mentioned in the human experiment study. Mouse spermatozoa parameters were estimated by assessment about 10 of randomly selected microscopically fields under a high power magnification of 40X objective [17].

➢ **Sperm Concentration:**

A drop of semen suspension is placed on a slide and covered with a cover slip, Sperm concentration per milliliter (ml) was reported from the mean number of spermatozoa in 10 random microscopically fields and

multiplying the mean number by a factor of one million[18]. Using powers microscopically fields under magnification of 40X. Concentration of sperms (sperm/ml) was calculated from the mean number of sperm in ten.

Sperm concentration (million/ml) = number of sperm × 10^6 [19].

- **Sperm Motility**: The sperm motility are percentage of sperm that moving. The freshly made; wet preparation is left to stabilize for approximately one minute. Motility estimation can conveniently be carried out at a room temperature 24°C [20]. The microscopic field is scanned systematically and the motility of each spermatozoon encountered is graded according to WHO (2010) as following:

- Progressive motility (PR): spermatozoa moving actively, either linearly or in a large circle, regardless of speed.
- Non-progressive motility (NP): all other patterns of motility with an absence of progression, e.g. swimming in small circles, the flagella force hardly displacing the head, or when only a flagella beat can be observed.
- Immobility (IM): no movement.

$$\text{Sperm motility (\%)} = \frac{\text{No. of motile sperms}}{\text{Total No. of sperms}} \times 100$$

- **Normal Sperm Morphology**:

Normal spermatozoon has an oval shaped head with a pale anterior part (acrosome 40-70% of the head area) and a darker posterior region. The length to width ratio of the head should be 1.50 to 1.75.the tail should be attached in a symmetrically situated fossa in the base of the head. The mid piece is first parts of tail. Categories of abnormalities in head this abnormalities classified primary abnormalities, amorphous head, tapered head, macrocephalic head and double head. Abnormalities in sperm tail classified secondary abnormalities as: bent tail or broken tail, coiled tail, short tail, double tail

[21]. The cytoplasmic droplet enlarge in midpiece refer defect in sperm maturity in the epididymis [22]. Percentage of abnormalities sperm morphology more than 70% classified as teratozoospermia and associated with infertility. At least 100 spermatozoa were counted and percent normal sperm morphology was reported according to the following formula:

Normal sperm morphology (%) = No. of normal sperm / total number of sperms X 100

- **Sperm Agglutination:**

Aggregation of sperm cell means that motile sperm cell stick to each other head to head, midpiece to midpiece and tail to tail or in a mixed way, e.g. midpiece to tail([23]. The presence of agglutination is suggestive of an immunological cause of infertility. The agglutination was assessed at the time of determination of sperm motility percentage (WHO, 2010). For estimation of percentage of sperm agglutination, the following formula is used:

Agglutinated sperm (%) = No. of agglutinated spermatozoa / totalNo. of spermatozoa X 100

Results:

- **Sperm concentration:**

Table 1 showed sperm concentration for male mice treated with different doses of metformin for six weeks presented in figure (4.3).Significant reduction ($P<0.05$) was obtained for all groups of treated males as compared to control group. However, no significant difference ($P>0.05$) were registered among all treated groups (G2, G3and G4).

- **Sperm Motility (A,B,C):**

Significant increment ($P<0.05$) was noticed in the percentage of sperm motility for treated groups (G3,0.4mg) as compared to other two treated groups (G2 and G4) and control group(G1).Similarity, significant increase ($P<0.05$) was assessed in the sperm motility treated group(84.3±6.00) when compared to treated group G2(80.65±3.65) . However, no significant difference ($P>0.05$) was observed between control group (G1) and treated group (G2). Also, no significant difference ($P>0.05$) was noticed between G1and G4.

- **Progressive sperm motility (grade A and B):**

In the present study ,highest and significant increase ($P<0.05$) was noticed in the percentage of progressive sperm motility for male mice of treated group(G3) as compared other two treated groups (G2andG4)and control group (G1) .In contrast, non significant differences ($P>0.05$) were assessed in the progressive sperm motility (%) when compared to treated groups(G2 and G4). Meanwhile, significant difference ($P<0.05$) was appeared between treated groups (G2 and G4).

- **Sperm motility grade A:**

In this study appeared significant decreased ($P<0.05$) was observed sperm motility grade A between G1 (45.05±8.08) with G2 (39.40±5.91), while significant increase ($P<0.05$) was associated control with G4

(51.95±13.18) highly statistical significant increase (p≤0.001) was noticed compare G3 (74.85±7.29)with control, G2 and G4.

Table 1: showed sperm parameter compared between control and treated groups and between treated groups.

Parameters	Control group	Low dose 0.2 mg	Mid dose 0.4mg	High dose 0.6 mg
Sperm concentration (million/ml)	25.06±5.64	14.44±2.89 a**	13.84±2.03 a**, bNS	12.58±1.65 a**, bNS, cNS
Progressive motility %	66.40±6.89	62.20±4.02 aNS	81.85±6.58 a**, b**	68.60±10.23 aNS, b*, c**
Grade A%	45.05±8.08	39.40±5.91 a*	74.85±7.29 a**, b**	51.95±13.18 a*, b**, c**
Grade B%	20.85±3.41	22.75±4.93 aNS	7.00±2.43 a**, b**	16.90±6.54 a*, b**, c**
Grade C%	16.30±4.05	18.50±2.86 aNS	6.40±3.78 a**, b**	15.45±5.31 aNS, b*, c**
Grade D %	17.80±6.00	19.35±3.65 aNS	11.75±5.87 a**, b**	15.70±5.60 aNS, b*, c*
Motility %	82.20±6.00	80.65±3.65 aNS	88.25±5.87 a**, b**	84.30±5.60 aNS, b*, c*
Abnormal morphology %	11.25±1.95	21.05±6.48 a*	1.75±0.69 a*, b**	55.15±24.39 a**, b**, c**
Agglutination	8.19±3.30	8.75±2.34 aNS	2.02±0.85 a**, b**	15.18±6.73 a**, b**, c**

-Sperm motility (grade B):

No statistical significant difference (P>0.05) was compared the control (20.85±3.41) with G1 (22.75±4.93), G2 (7.00±2.43) highly statistical significant decrease (p≤0.001)was compared with G1, G2 and G4. G4 (16.90±6.54) statistical significant decrease (p≤0.05) was compared with G1, highly statistical significant decrease (p≤0.001) was compared with G2, while Highly statistical significant increase (p≤0.001) was compared within G3.

-Sperm motility (grade C):

In the current study, shows sperm motility (grade C), statistical non-significant difference (p>0.05) was compared G2 (18.50±2.86) with G1 (16.30±4.05). G3 (6.40±3.78)

highly statistical significant decrease ($p \leq 0.001$) was compared to G1 and G2. G4 (15.45±5.31) significant decrease was compared to G2, while highly statistical significant increase ($p \leq 0.001$) was compared to G3.

- **Sperm Motility (grade D):**

Non-significant difference ($p > 0.05$) was compared G2 (19.35±3.65) with G1 (17.80±6.00).G2(11.75±5.87) highly statistical significant decrease ($p \leq 0.001$) associated with G1 and G2,G4 (15.70±5.60 non-significant difference ($p > 0.05$) was compared to G1 and G2 were as significant increase was compared to G3.

- **Abnormal Sperm Morphology (%):**

Abnormal sperm morphology(%) for male mice treated with difference doses of metformin for 45 days presented in figure (4.7) appeared significant increase ($p \leq 0.05$) was compare d G2 (21.05±6.48) with G1 (11.25±1.95). G3 (1.75±0.69) significant decrease ($p \leq 0.05$) was compared to G1 and highly significant increase ($p \leq 0.001$) was compared to G2 ,G4 (55.15±24.39) significant increase ($p \leq 0.001$) was compared to G1, G2 and G3.

- **Sperm Agglutination:**

Statistical non-significant difference ($p > 0.05$) associated between G2 (8.75±2.34) with G1 (8.19±3.30). G3 (2.02±0.85) highly statistical significant decrease ($p \leq 0.001$)was compared to G1, G2 and G4,G4 (15.18±6.73) significant increase ($p \leq 0.001$) was compared to G1, G2 and G3.

Discussion:

In the present study, different doses of metformin were applied according to physiological human used doses. Metformin is regarded a safe drug and side effects minimum,

treatment of choice for T2D and overweight [24].Therefore insulin sensitivity is improved by hyperglucose uptake and utilization [25]. Furthermore, metformin is decreasing reactive oxygen species which improve epithelial cells and reproductive tissues, metformin improve the activity of Sertoli cells [26]. Metformin treatment for 6 week orally was appeared non-significant difference ($P \geq 0.05$) in the whole body weight among different groups in this study. These results in agreement with other study [27]. They administered MF to diabetic patients non depending on insulin. Another study done by Ting and his college [28] certified same current result in this study. In spite of them administered MF combined with vitamin B12. While, others referred to loose of animal weight as well as decrease size of most studied organs in diabetic - induced rat when administered MF only. The explanation for these present results through mechanism of MF action by improve sensor of insulin leading to increase glucose metabolism [29]. Results of the present study ware demonstrated a significant decrease ($P<0.05$) in the weight of testis for treated groups when compared to the control group. These results agreement with Oliveira [32] study, which observed a significant decrease ($P<0.05$) in the testicular weight of diabetic rabbits administered MF (120 mg/kg body weight). Another studies suggested that metformin may inhibit testicular growth [30] [31]. They observed significant impact on the development of the male reproductive tract when using MF. However, effects of metformin on male reproduction reported recently by several studies appeared that the deregulation of insulin is detrimental to the male reproductive system as it modulates by inhibition of lactate production [33]. While other study noticed improvement in the human

reproductive system when given 50 Mm and 500Mm of MF. These two doses considered nontoxic and have no side effects on reproductive system in human. While tartarin and co-workers [33] showed higher sensitivity of human testis to MF more than mice testis. Furthermore, MF causes decrease testis size and weight. More recently, testosterone production decrease and reduction size of Sertoli cells combined with reduction of testicular size. This refers to effect of metformin on the physiological functions for Sertoli cells to regulate germ cell development. Consequently, germ cells was decreased to a limited number and a significant number of germ cells die by apoptosis [34] . In the current study, the sperm concentration showed highly significant reduction ($P<0.01$) for treated groups when compared to control group. This result in agreement with another study [29], who certified a highly significant decrease in sperm cell count compared among treated groups with control group in the rabbits. Another study done by Adaramoye *et al*. [34]. They recorded that the MF interfere with normal testicular physiological function leading to spermatogenic failure leading to decrease sperm concentration in rats.This study was appeared that the sperm motility decreased non significantly ($P>0.05$) when using low dose (0.2 mg MF/male mouse/day), while mid dose (0.4 mg MF/male mouse/day) demonstrated highly significant increase when compared with control and high dose (0.6 mg MF/male mouse/day) . On the other side, high dose showed no significant increase ($P>0.05$) associated with controls. These results regarded to low dose in agreement with another study done on rabbits [32] and on rats [34]. They mentioned two causes when using MF including increase glucose metabolism and reduce ROS production. In contrast, a recent

study observed reduced sperm motility in rabbits administered 5000Mm MF[34]. This result in agreement with low dose while disagreement with mid and high dose used in the present study. Results in this study showed highly significant increment (P<0.01) in the percentage of immotile sperm for low dose when compared with controls. While highly significant decrease (P<0.01) in the immotile sperm (%) for mid dose when compared with low dose and control groups. However, high dose appeared non-significant decrease (P>0.05) as compared to control group and significant decrease when compared with low dose, while non-significant increase (P>0.05) as compared to mid dose. Results of the low dose agreement with study [32] who observed that the rabbits treated with MF recorded highly significant increase (P<0.01) in the immotile sperm. Another study in agreement with results current study in regard to low dose [34]. They referred a decrease in the Sertoli cell population was due to a decrease in cell proliferation and increase cell death. Consequently, Sertoli cell may be decrease support of germ cells, therefore, high number of germ cells die by apoptosis as certified by histological study[34]. It appeared an agreement with the present results of mid dose which recorded highly significant decrease (P<0.01) in the percentage of immotile sperm. The percentage of abnormal sperm morphology (ASM), in this study, showed 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. Results of low and high doses in

agreement with study done on rabbits [32].

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