Effect of Curcumin on Sperm Parameters in Alloxan Induced Diabetes in Mice

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***Abstract*—Diabetes mellitus is a metabolic disorder that negatively affects different body organs. Induced type1 diabetes mellitus (T1DM) leads to hyperglycemia that if chronic might affect spermatogenesis and nuclear content of sperm in male mice. In the current study, diabetes was induced in male albino mice by a single i.p. injection of Alloxan (120mg/kg). For controlling diabetes, metformin (200mg/kg/day) and /or curcumin (100mg/kg/ day) were administered orally for 35 days. Male mice (n=40) were divided equally into five groups. Control, Induced Diabetes mellitus (DM), Diabetic treated with curcumin (DM+C), Diabetic treated with metformin (DM+M), Diabetic treated with curcumin and metformin (DM+CM). The results of the current study showed that curcumin and metformin reduce blood glucose levels ($p<0.001$), and decrease pancreatic and testicular damage in diabetic mice. Also, metformin alone or in combination with curcumin reduces the rate of abnormalities and DNA damage of sperms at the end of the experiment ($p<0.05$). In conclusion, curcumin with metformin has a beneficial effect in reducing abnormalities and DNA fragmentation of sperm. Further study to investigate the effect of curcumin and metformin on post fertilization outcome in diabetic mice is suggested.**

***Keywords* — Diabetes, Curcumin, Metformin, Sperm, Mice**

INTRODUCTION

Diabetes mellitus (DM) is a common chronic metabolic disorder resulting from pancreatic damage that leads to insulin disturbance and fluctuation of blood glucose. The persistent elevation of hyperglycemia resulted in a series of toxic effect in tissues including oxidative stress, upsurge polyol pathway, advanced glycation end-products (AGEs), increase hexosamine biosynthetic pathway (HBP), which affects different body organs (1).

Induced Type1 diabetes mellitus (T1DM) in laboratory animal had widespread practice in the field of scientific research. The use of either Alloxan or Streptozotocin (STZ) to damage pancreatic beta cells is a well-known practice that is commonly used to induce T2DM in laboratory animal (2). However, it has been suggested that Alloxan induce diabetes with less painful neuropathy than STZ in rats (3).

Metformin is a frequently used medication for the treatment of diabetes in human. This acts through activation of Adenosine Monophosphate kinase (AMPK) that inhibits gluconeogenesis and promote glycolysis (4). Therefore, several studies had used metformin to control diabetes in mice and rats (5-7)

On other hand, Curcumin, extracted from natural plant Curcuma longa, is widely used for its antioxidant and anti-inflammatory effect (8-10). Also, administration of curcumin improves diabetic status in obese patients (11).

In their study, (12) demonstrated that curcumin is beneficial when used in the appropriate dose and higher doses negatively affects antioxidant and vitamin C levels.

Scarce evidence investigates the effect of curcumin in combination with metformin on sperm parameters in diabetic mice. The current study is performed to evaluate the effect of both curcumin and /or metformin on sperm viability, normal morphology and the degree of DNA damage of diabetic mice sperm.

MATERIALS AND METHODS

Animals

The experiment was conducted in the animal house unit at the Biotechnology research center / University of Al-Nahrain. And all procedures and ethics were approved by the scientific committee in the center (Ethical Approval no.E.B.18). In the current study, total of 40 adult male albino mice (8-10 weeks old) were divided equally into 5 groups that acclimated for two week before conducting the experiment. Control negative with no treatment, Diabetics (injected with single dose of Alloxan (120mg/kg B.W)) that received no treatment (DM). Diabetics treated with 100 mg/kg curcumin (DM+C). Diabetics treated with 200mg/kg metformin (DM+M). Diabetics received 100mg/kg curcumin and 200 mg/kg metformin. All animal were supplied with water and fed ad libitum for 35 days. Mortality rate were monitored during the study period

Chemicals

All chemicals were purchased from sigma Aldrich unless otherwise indicated. Metformin (Glucophage® 1000mg) were purchased from MERCK Sante S.A.S – Franta.

Depending on the study by (13), the equivalent dose was calculated for the current study medication and as follows:

X=(human dose (mg)*average adult mice weight (gm))/(average adult human weight (gm))*Human to mice dose factor

Where, initial human dose is 1000 mg/day for metformin; and 500 mg/day for curcumin

Average adult human weight is 60 kg (60,000 gm), and average mice weight in this study was 25gm, Human to mice dose factor is 12.3. So, calculated metformin dose in mice of this study = 5.125mg for mice weighted 25gm which means 205 mg/kg, and calculated curcumin dose =2.5625mg which means 103 mg/kg

In the current study, metformin was given orally via gavage daily in a dose of 200 mg/kg; and curcumin was given orally via gavage daily in a dose of 100mg/kg

Induction of diabetes

Fasting period of around 15 hrs prior induction of diabetes in mice was applied. Diabetes was induced via a single intra-peritoneal (i.p.) injection of Alloxan solution (dissolved with normal saline) at a dose of 120 mg/kg of body weight. Fasting blood glucose level was first measured 72hrs of Alloxan injection and once a week then after. Only hyperglycemic mice with blood glucose level > 200 mg/dl were included in diabetic groups of this study.

Sperm preparation

After 35 days of study period, the cauda epididymis was dissected in petri dish with 1 mL of pre-warmed physiological solution to release spermatozoa. The dishes were placed on heated stage at 370C during processing of sperm and sperm analysis (14).

Sperm analysis

The percentage of viable sperms and abnormal morphology of spermatozoa were assessed using Eosin-Nigrosine staining protocol and as previously described by (15) with modification. Briefly, after sperm preparation, 50µl from sperm suspension was placed on a glass slide and two drops of 1% eosin added and left for 20 seconds. Then three drops of 5% nigrosine were added and left for 20seconds. After that, 20µl of the mixture was placed on a new slide and thin smear was prepared. All semen smears were allowed to dry at room temperature and examined under light microscopy at ×400 magnification.

Sperm DNA integrity

DNA integrity was assessed by chemical techniques using Acridine Orange (AO). Acridine Orange dye was purchased from Sigma Aldrich (St Louis, MO, USA). Briefly, a smear of sperm samples was prepared on slides and let to dry at room temperature (RT). Then, the dried smears were fixed in a ratio 3:1 of methanol: glacial acetic acid, at 4 oC for 2 hrs. After that, a freshly prepared AO stain was mounted on slides and covered the area of sperm smears for 10 min. (AO stain was prepared by dissolving 0.19mg/ml of stain in phosphate-citrate buffer with final pH=4). Slides then gently washed with distilled water and allow drying at RT. The smears were examined directly on the same day of preparation using fluorescent microscope (Zeiss Co., Germany).

Acridine orange display the degree of nuclear DNA affinity to denaturation and the distinction between normal sperm DNA (green) and the abnormal denatured single stranded DNA(yellow or orange to red).

Histopathological examination

Histopathological examination was performed at the end of the experiment using Hematoxylin and eosin staining and as previously described by (16,17). The pancreatic and testicular tissues were used to evaluate the degree of damage of Alloxan before and after administration of curcumin and or metformin.

Statistical analysis

Statistical analysis was performed by Minitab software version 18 and using Anova and t-test to find the significance of the differences between study groups. The standard deviation and standard error were presented and differences between groups were considered statistically significant at $p<0.05$.

RESULT & DISCUSSION

Blood glucose

Fasting blood glucose level was significantly increased in DM group compared with control ($p<0.001$). The administration of metformin at 200 mg/kg reduce blood glucose and mortality rate in diabetic mice. Also, curcumin at 100mg/kg alone or in combination with metformin significantly reduce blood glucose in diabetic mice as shown in Table (1).

Table 1: Blood glucose measurements mg/dl (Mean± SD) prior induction of diabetes in study groups

Groups	Day 3	Day 10	Day 17	Day 24	Day 31
Control	105.13± 10.55	111.00± 13.83	106.88± 14.84	109.38± 16.76	113.88± 12.80
DM	389.00± 83.07**	326.67± 64.29**	280.00± 7.07**	283.5± 4.95**	274.00± 5.66**
DM+C	394.38± 70.48	275.50± 57.74	150.83± 13.57**	149.17± 10.21**	142.50± 6.89**
DM+M	402.25± 117.4	327.71± 74.49	214.86± 47.70*	156.14± 18.86**	133.86± 16.66**
DM+CM	349.38± 66.89	235.50± 48.35*	168.63± 16.16**	143.75± 19.41**	127.88± 16.23**

* Statistical significance at $p<0.001$; ** Statistical significance at $p<0.05$

Six mice were died in DM group that received no treatment, while two and one died in curcumin and metformin groups respectively. Furthermore, no mortality in DM+CM group was occurred Except DM+CM group that show low blood glucose level at day10, glucose level was noticeably decreased in curcumin or metformin groups after day10 from administration of Alloxan (see Figure 1).

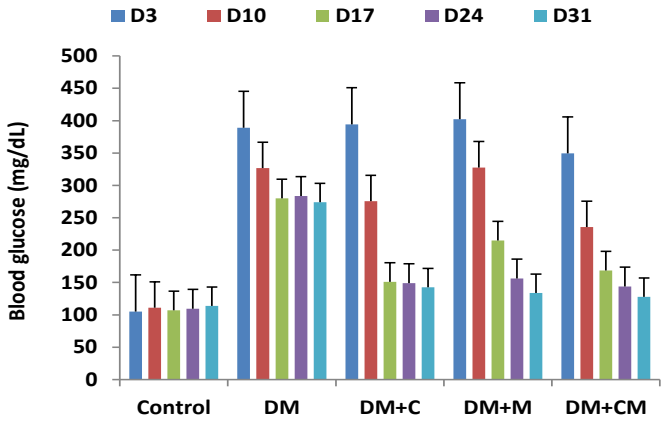

Figure 1. Blood glucose level in mice (Mean± SE) in experimental study groups in different timing point

Sperm parameters

Depending on microscopic evaluation and staining protocols (Figure 2-A-C), the results of the current study reveals that administration of metformin alone or in combination with curcumin reduce ($p<0.05$) the percentage of abnormal sperm compared to DM group.

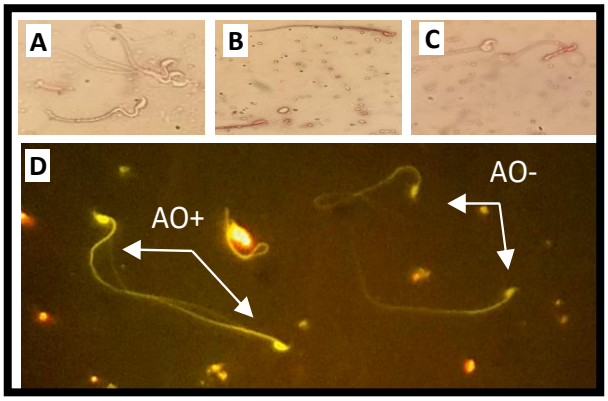

Figure 2. The Morphology of mice sperm prior 35 days of the experimental study. A, Live sperms; B, Dead sperms; C, Abnormal sperms; D, Acridine orange negative in normal sperm (AO-) stained green fluorescence and Acridine orange positive in abnormal denatured sperm DNA(AO+) stained yellow to orange-red fluorescence.

The sperm DNA damage where reduced noticeably after treatment with curcumin and or metformin. However, the viability of sperm did not affected after induction of diabetes, as shown in Table 2, and Figure 3.

Table 2. Percentage of live, abnormal and DNA damage of sperm (Mean± SD)

Groups	Live sperm %	Abnormal sperm %	Sperm DNA damage
Control	76.88± 4.96	20.63± 5.94	11.13± 5.64
DM	67.50± 3.54	32.50± 3.54*	30.00± 7.07**
DM+C	70.00± 5.24	22.50± 7.75*	19.00± 4.73*
DM+M	71.43± 5.56	21.43± 6.90*	18.29± 4.42*
DM+CM	73.75± 5.30	21.88± 5.82*	15.50± 6.57*

* Statistical significance at $p<0.001$; ** Statistical significance at $p<0.05$

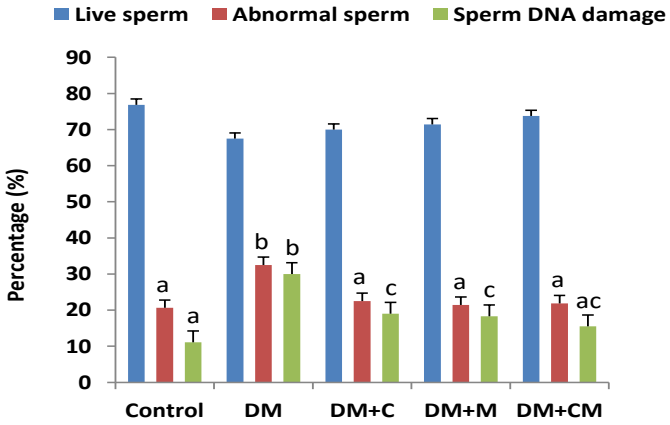

Figure 3. Percentage of live sperm, abnormal sperm and DNA damage of sperm (Mean± SE)
Different superscript letters indicate statistical significance.

Histopathological examination

Pancreas

The results of the current study showed the presence of interlobular infiltration of Mononuclear cells (MNCs), and intravascular hemolysis with abnormal morphology of Langerhans' islets in diabetic group compared with control. However, these effects were reduced in curcumin and or metformin groups as shown in Figure (4).

Testis

In this study, histological changes in the samples of testis were observed. These include the changes in the morphology of seminiferous tubules and degeneration in testicular cells of diabetic mice. The administration of curcumin with metformin ameliorate to certain extend the damage in testicular tissue, see Figure (5).

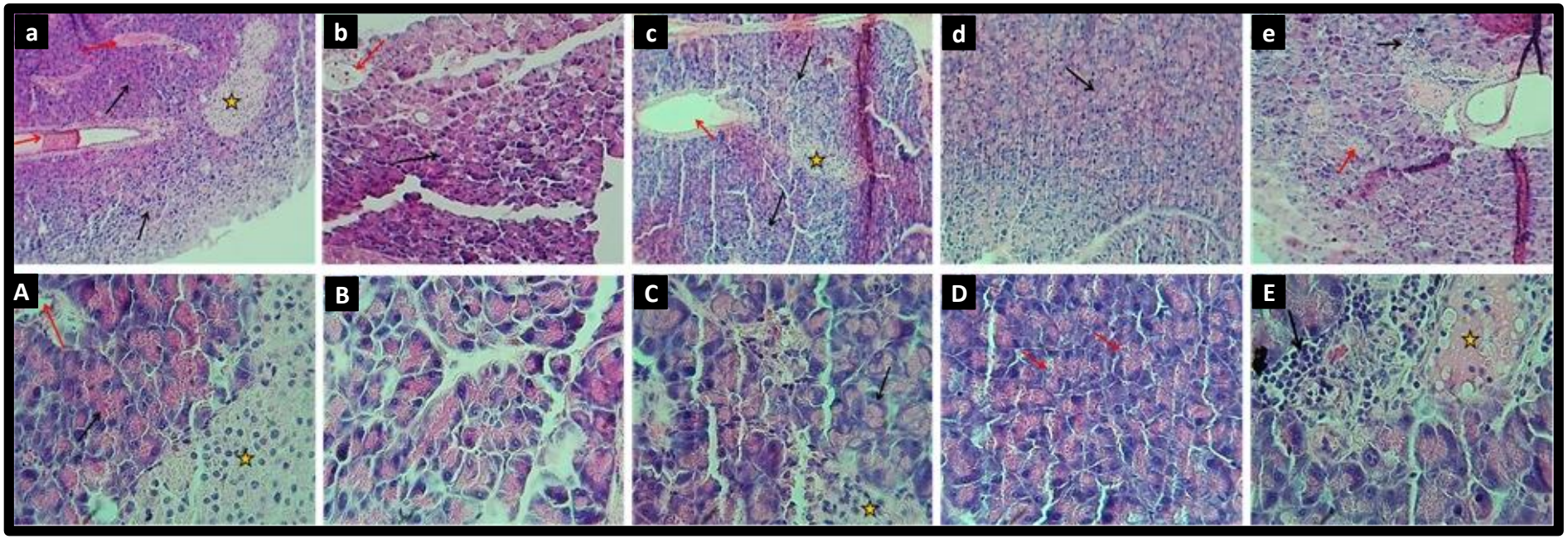

Figure 4. Histological morphology of pancreas in normal control (a, A), Alloxan Induced Diabetic mice (b, B), diabetic mice treated with curcumin (c, C), diabetic mice treated with metformin (d, D), and diabetic mice treated with curcumin and metformin (e, E). Hematoxylin & Eosin stain and under magnification of 100x (a, b, c, d & e) and 400x (A, B, C, D & E). Note the blood vessels (Red arrow), pancreatic acinar cells (Black arrow), and islets of Langerhans (Asterisks).

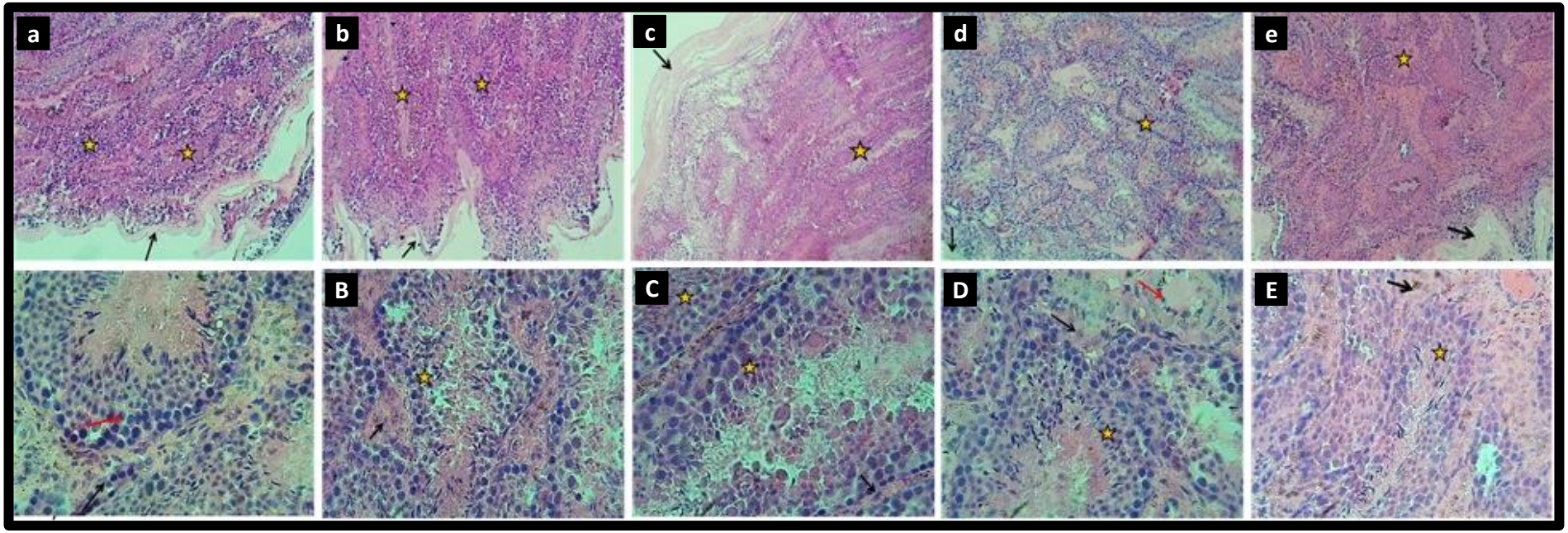

Figure 5. Histological morphology of tests in normal control (a, A), Alloxan induced diabetes (b, B), diabetic mice treated with curcumin (c, C), diabetic mice treated with metformin (d, D), and diabetic mice treated with curcumin and metformin (e, E). Hematoxylin & Eosin stain and under magnification of 100x (a, b, c, d & e) and 400x (A, B, C, D & E). Note: germinal epithelium (Red arrow), Tunica albuginea (Black arrow), Interstitial cells (White arrow), and seminiferous tubules (Asterisk).

In the current study, administration of curcumin alone or in combination with metformin ameliorates blood glucose level after induction of diabetes in mice. In previous study, it was speculated that this might attributed to the effect of curcumin on lowering plasma free fatty acids that decrease the production of glucose in the liver of diabetic rats (18). The fluctuation of blood glucose in experimental groups might due to the timing of fasting period before glucose reading intake (19, 20).

On other hand, our results reveal no effect of inducing diabetes on sperm viability (p=0.070). However, this disagrees with the findings of previous study (14). It might be possible that in large scale study this tendency being changed and the statistical difference increase between DM and control group.

Curcumin and or metformin decrease the rate of abnormal sperms and degree of sperm DNA damage. Also, to certain extent the histological changes in testicular tissue were restored after administration of curcumin and metformin. These results are in line with previous studies in rats (21-23). It has been suggested that induced diabetes increase oxidative stressors that affect negatively testicular tissue in rats, which restored by administration of curcumin (24).

CONCLUSION

Despite the survival of few numbers of animals in DM group, lowering blood glucose with curcumin and metformin rescue mice and decrease the effect of diabetes on pancreatic and testicular tissue. These in turn ameliorate the reproductive efficiency in diabetic mice. Future study to investigate the effect of curcumin and metformin on fertilization outcome is advised.

Declaration of interest

The authors declare that there is no conflict of interest.

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