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Physiological disturbances and histological damages of reproductive system in male rats resulted by metformin

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ABSTRACT

The current study was designed to evaluate the effects of metformin in some physiological and histological parameters of reproductive system in the diabetic male rats experimentally. Forty rats were randomly divided into four groups. The (control) first group was treated with normal saline; the second was infected group which included rats diabetic experimentally treated by alloxan; the third and fourth groups were induced diabetic experimentally and received metformin suspension (150 mg kg-1 daily) at one and two doses respectively. Twelve hours was time between these two doses. Reproductive ability was measured by determination of some physiological parameters as count sperm, malformations, motility, dead sperm, weights of sex organs (testes, epididymis, prostate glands and seminal vesicles) and the damaged testicular structure. The present results indicated that the administrated metformin at high doses led to significant decrease ($p < 0.05$) in total count of sperm, motility and in weights of all sex organs such as testes, epididymis, prostate glands and seminal vesicle, while significant increase ($p < 0.05$) in sperm abnormalities and dead sperm. Also, the results showed that the diabetes and exposure to metformin caused many malformations of sperm such as shattered sperm, the lacking, hooked and quirky tail, while the abnormalities of head included the lacking, Globozoospermia and elliptical head. The histological damages in testicular structure were observed including the destroyed and raised thickness of connective tissue among seminiferous tubules, necrosis and reduction numbers of spermatocytes along with the congestion of blood vessels. Many histological alterations occurred clearly due to treated with high dose of metformin after diabetic experimentally including the most affected structural troubles as large fracture of seminiferous tubules, expansion of bloody congestion and raise of necrosis in testicular structure.

Keywords: Diabetes mellitus, Metformin, Rats, Reproductive system.
Article type: Research Article.

INTRODUCTION

Diabetes mellitus is a serious disease which characterized by increasing concentration of glucose in blood and flowing of sugar in urine of patients (Ugbenyen *et al.* 2009). It is a chronic disease that resulted from deficiency, resistance of insulin or both (Broadhurst *et al.* 2000). Polyuria, losing of body weight, thirst, haziness of vision, atherosclerosis and cardiovascular disease were common symptoms related with diabetes (Saravanan & Pari 2006). Other complications which associated with diabetes mellitus included neuropathy, nephropathy and retinopathy (Kamal *et al.* 2012). Metformin is a compound belonging to biguanids which considered a first-line treatment for type 2 diabetes (Dujic *et al.* 2016). It is used for management of diabetes and control on their complications, in addition to exercise or diet (Manonmani & Manimekalai 2018). Metformin has important role as anti-hyperglycaemic effect. This activity is due to its ability to inhibit glycerol phosphate dehydrogenase of mitochondria leading to decline of glucose (Standle & Schnell 2012; Madiraju 2014). The previous studies found that using metformin at dose of 0.5-2 mL day^{-1} results in non-infectious damages as vomiting, nausea, diarrhoea, abdominal pain and appetite losing (Neha *et al.* 2013). There are many studies of biology and physiology of mice and rats in the world (Hazratian 2017; Qasim Alkafajy 2022; Assi *et al.* 2022). However, few reports about diabetes

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in these animals (Humaidan Al-Moussawi 2022). Hence, the current study was aimed to investigate influence of metformin in some physiological and histological parameters of reproductive system in male rats diabetic experimentally.

MATERIALS AND METHODS

Induction of diabetes in rats

Diabetes was induced experimentally in rats by intraperitoneal injection at single dose (150 mg kg^{-1} body weight) of alloxan which dissolved in 0.9% normal saline. Diabetes occurred after one week from injection (Alarcon-Aguilara *et al.* 2002).

Laboratory animals and experimental design

Forty adult male rats, *Rattus norvegicus* were used in current experiment. Rats were 11-13-week olds and 220-260 g in weight. The animals were obtained and housed in standard cages at the animal house of Biology Department, University of Thi-Qar, Iraq. They were kept in 21-24 °C and 12:12 h light: dark cycle. The animals were divided into four groups as follows:

First group (Control; n = 10): Rats were administrated normal saline (0.5 mL daily) by intraperitoneally injection.
Second group (Diabetic): Diabetic rats experimentally by alloxan.
Third group: Rats were induced diabetes experimentally and administrated metformin suspension (150 mg kg^{-1} body weight daily; Majithiya & Balaraman 2006).
Fourth group: Rats were induced diabetes experimentally and administrated metformin suspension with two doses (150 mg kg^{-1} body weight daily) in each dose and 12 h was time between these two doses (Majithiya & Balaraman 2006). The experiment was prolonged for four weeks and all the animals were treated with metformin by orally method.

Sperm Characteristics

Sperm count was calculated according to Soto (1983); method of Wyrobek and Bruce (1975) was used to determine the rate (%) of the sperm abnormalities. Sperm motility was assessed according to Robb *et al*. (1978) while the dead sperms was evaluated according to Chemineau *et al*. (1991).

Measurement of the organ weights

After four weeks of the experiment, the sexual organs were weighted by sensitive electronic balance and the weights compared with control group and among all groups. These organs included testes, epididymis, prostate glands and seminal vesicles.

Histopathological study

The method of Bancroft and Gamble (2008) was used to prepare the histological sections of testes. At first, testes were fixed in 10% formalin, dehydrated in ethyl alcohol, then xylene was used for clearing, then embedded in paraffin wax. Sections were stained with haematoxylin and eosin. The rotary microtome was used to prepare sections by 6 micro-meter thick.

Statistical analysis

The data are presented in tables as mean ± standard deviation, analysed by SPSS (version 21). Significant occurred among means, was set at the level of $p < 0.05$ by LSD (George and Mallery 2011).

RESULTS AND DISCUSSION

The characteristics of sperm were depicted in Table 1 exhibiting a significant decrease ($p < 0.05$) in the count and motility of sperm in diabetic groups and groups treated with metformin at one and two doses, in comparison with control group. Significant decline ($p < 0.05$) in these parameters was also observed in groups receiving metformin, compared to diabetic group. Moreover, the results showed increased significantly ($p < 0.05$) in sperm abnormalities and dead sperm of infected group (diabetic group) and also groups which were administrated metformin, compared

to control group. The significantly ($p < 0.05$) increased malformations and dead sperm occurred in groups which received metformin, compared to diabetic group.

Table 1. Effects of metformin in some sperm characteristics (n = 10; mean ± standard deviation).

Group	Sperm Count ($\times 10^4$)	Sperm Abnormalities (%)	Motility (%)	Dead Sperm (%)
Control group	$210.08^{a} \pm 1.43$	$7.77^{d} \pm 0.93$	$80.42^{a} \pm 1.39$	$15.54^{d} \pm 0.57$
Diabetic group (Infected group)	$179.92^{b} \pm 0.90$	$35.98^{c} \pm 0.84$	$64.84^{b} \pm 0.80$	$21.58^{c} \pm 0.68$
Diabetic group + Metformin (One dose)	$143.35^{c} \pm 1.22$	$59.05^{b} \pm 0.68$	$52.69^{c} \pm 1.02$	$29.56^{b} \pm 0.40$
Diabetic group + Metformin (Two doses)	$121.51^{d} \pm 0.93$	$88.88^{a} \pm 0.96$	$46.18^{d} \pm 0.61$	$39.72^{a} \pm 0.91$
L.S.D	0.55	0.42	0.48	0.32

Note: Different letters indicate to significant difference ($P < 0.05$) among groups.

These results may be related to oxidation and production of free radicals (ROS) which resulted from diabetes mellitus. ROS have destructive effect on epithelial tubular and depression of spermatocytes led to decline in count of sperm. This is in agreement with Ahmed (2005) who reported that oxidation, free radicals generation and descending of antioxidant defences caused by diabetes. Sajal *et al*. (2008) found the induction of apoptosis in spermatocytes by ROS leading to the decreased sperm count. These alterations in the sperm parameters may be linked to apoptosis which occurs concurrently with diabetes. This result is in agreement with Alves *et al*. (2013) who suggested that glucose toxicity causes damages in testicular tissue by apoptosis and autophagy. The decreasing in sperm and elevated of its abnormalities may be regarded to diabetes. Oishi *et al*. (2004) observed testicular dysfunction and spermatogenetic injury occurred by diabetes mellitus. The lowering of sperm may be in association with abnormal autophagy due to diabetes. This finding is in line with Sato *et al*. (2016) who demonstrated that the excessive autophagy led to destroy of germ cells and subsequently decreasing of count sperm. Xiao *et al*. (2016) exhibited the hyperglycaemia led to non-activated of autophagy which is related to gene 4 (ATG4) by oxidation. All the previous changes of sperm characters in metformin groups did not accepted with Liu *et al*. (2019) who suggested the role of metformin in ameliorates of histological damages in diabetic rats induced by streptozotocin. In the present study, The rate (%) of abnormalities in the sperm, including shattered sperm, malformations in head of sperm as Globozoospermia, elliptical and lacking head, also damages in tail of sperm such hooked, quirky tail and lacking of tail (Figs. 2-8) were occurred in diabetic and metformin groups, increasing significantly ($p < 0.05$). This could be due to the effects of diabetes and metformin on testosterone activity which affect spermatogenesis. William (2000) observed that disturbances of spermatogenic processes were correlated with decline of gonadotropins. So, drop in testosterone causes reduction in sperm and elevated abnormalities. This is in line with Tartarin *et al*. (2012) who described dropping in testosterone level during metformin exposure.

Table 2. Effect of Metformin on weights the organs of the rat reproductive system (n = 10; Mean ± Standard deviation).

Groups	Testes	Epididymis	Prostate Glands	Seminal Vesicles
Control group	$1.38^{a} \pm 0.51$	$0.67^{a} \pm 0.00$	$0.57^{a} \pm 0.00$	$0.59^{a} \pm 0.01$
Diabetic group (Infected group)	$1.31^{b} \pm 0.01$	$0.60^{b} \pm 0.01$	$0.52^{b} \pm 0.01$	$0.53^{b} \pm 0.01$
Diabetic group + Metformin (One dose)	$1.05^{c} \pm 0.00$	$0.44^{c} \pm 0.00$	$0.41^{c} \pm 0.00$	$0.41^{c} \pm 0.00$
Diabetic group + Metformin (Two doses)	$0.80^{d} \pm 0.01$	$0.33^{d} \pm 0.00$	$0.30^{d} \pm 0.00$	$0.34^{d} \pm 0.00$
L.S.D.	0.12	0.00	0.00	0.00

Note: Different letters indicate to significant difference ($P \leq 0.05$) among groups.

Table 2 depicted a significant decreasing ($p < 0.05$) in weights of male sex organs (testes, epididymis, prostate gland and seminal vesicles) in diabetic group compared to control group as well as significant declining ($p < 0.05$) in the metformin groups (one and two doses) compared to diabetic group which may be due to decline in the testosterone level by induction of diabetic and also administration of metformin. Chowdhury & Steinberger (1976) revealed the role of testosterone in the weight raise of testes and seminal vesicles. This is in agreement with Tartarin *et al*. (2012) who explained that decrement the testosterone is linked to administration of metformin, or this result is due to the decline of seminiferous tubular fluid, that contribute in sex organ weights especially testes (Gosh *et al*., 1992). The histopathological damages occurred in testes of diabetic and metformin groups included the destroyed interstitial tissue, simple necrosis, reduction of spermatocytes, the increased thickness of connective tissue, congestion, necrosis of spermatocytes and reduction of sperm (Figs. 10-16). These may be in association

with diabetic experimentally and treatment with metformin by oxidation and production of ROS. Mitra *et al*. (2013) observed histopathological alterations in testicular structure resulted from ROS. The destroyed interstitial tissue, necrosis, and congestion may be associated with oxidative stress and generation of ROS. This is similar to Ahmed (2005) who suggested oxidative stress and free radicals resulted by diabetes. Also, the reduction of spermatocytes belongs to the role of diabetes in free radicals generation, which is in line with Mohamed *et al*. (2009) who reported the destroyed tubular epithelium and contracting of spermatocytes caused by free radicals.

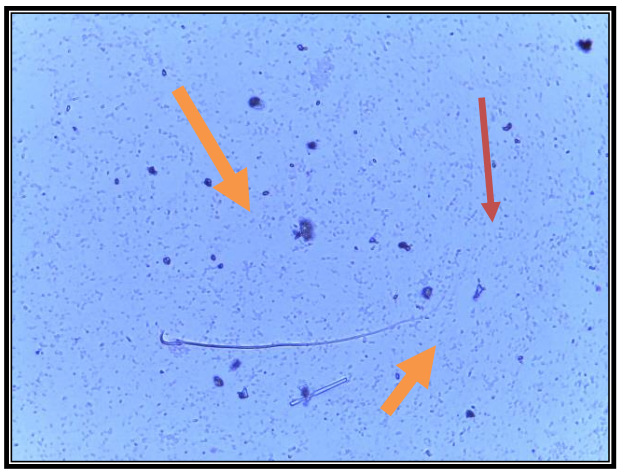

Fig. 1. Normal sperm in control group (Eosin Stain; 100 X).

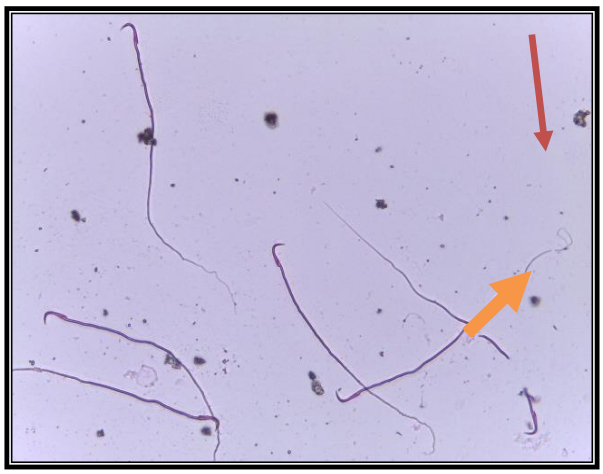

Fig. 2. Abnormalities of sperm (shattered sperm) in diabetic group (Eosin Stain; 100 X).

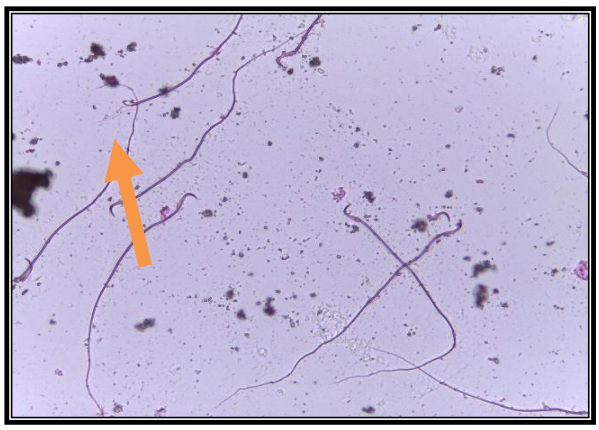

Fig. 3. Exhibiting abnormalities of sperm (Globozoospermia) in diabetic group (Eosin Stain; 100 X).

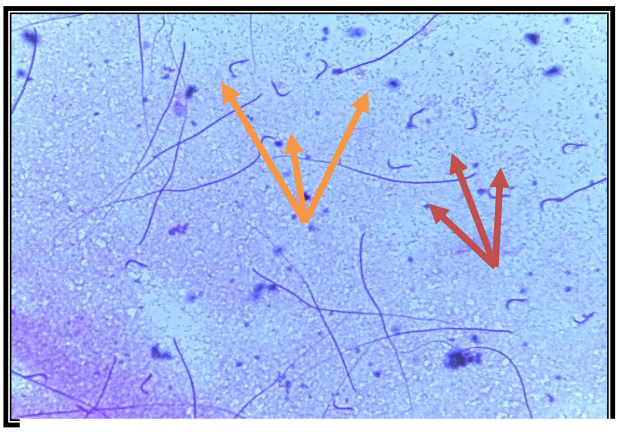

Fig. 4. Displaying abnormalities of sperm (lacking head and lacking tail) in diabetic group + one dose of metformin (Eosin Stain; 100 X).

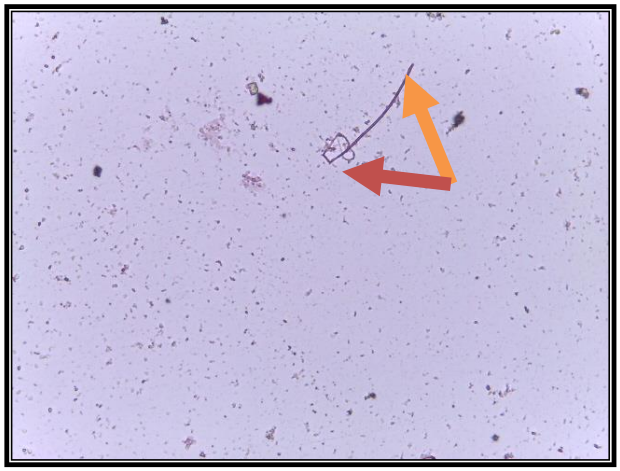

Fig. 5. Illustrating abnormalities of sperm (lacking head and hooked tail) in diabetic group + one dose of metformin (Eosin Stain; 100 X).

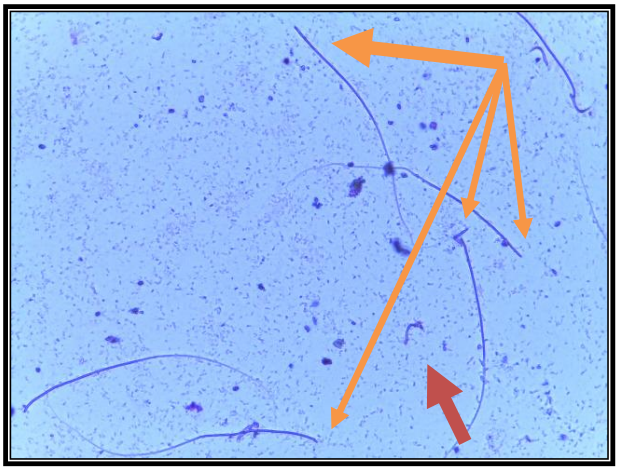

Fig. 6. Indicating abnormalities of sperm which most affected (lacking head and lacking tail) of diabetic group + two doses of metformin (Eosin Stain; 100 X).

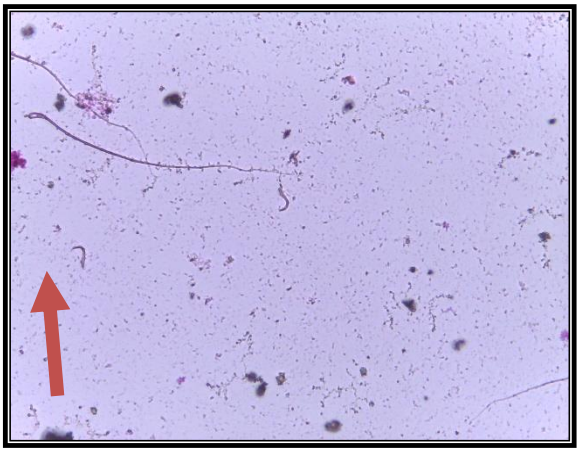

Fig. 7. Showing abnormalities of sperm (elliptical head) in diabetic group + two doses of metformin (Eosin Stain; 100 X).

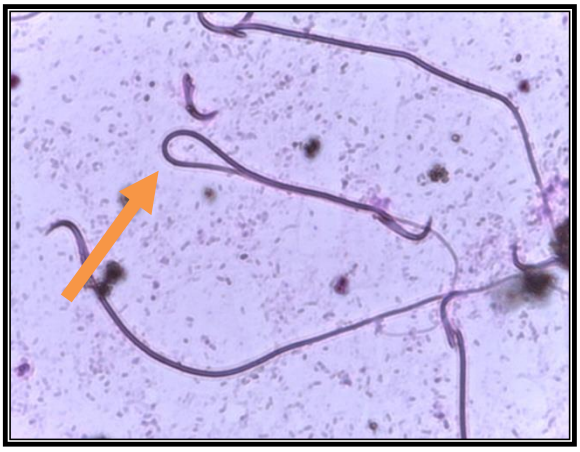

Fig. 8. Revealing abnormalities of sperm (quirky tail) in diabetic group + two doses of metformin (Eosin Stain; 100 X).

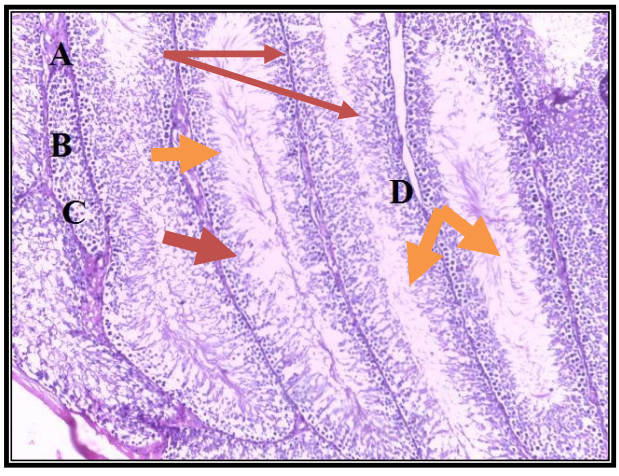

Fig. 9. Section in testis of control group showing seminiferous tubules (A) interstitial tissue (B) spermatocytes (C) sperms (D); (H&E; 100 X).

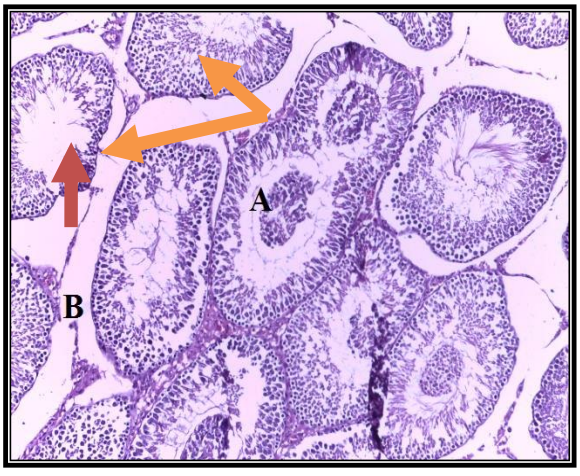

Fig. 10. Section in testis of diabetic group showing destroyed interstitial tissue (A) simple necrosis (B); (H&E; 100 X).

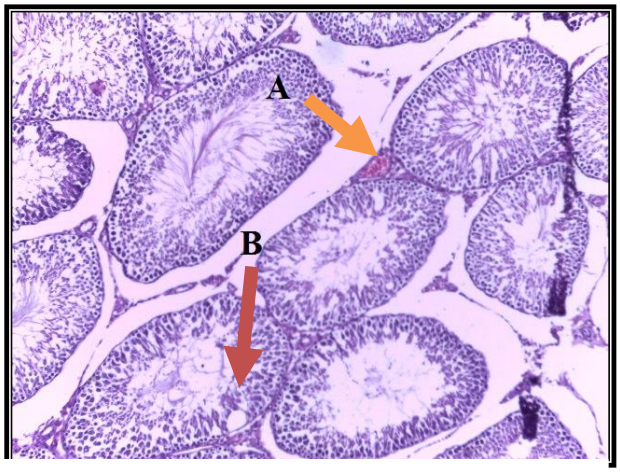

Fig. 11. Section in testis of diabetic group showing simple congestion (A); reduction of spermatocytes (B); (H&E; 100 X).

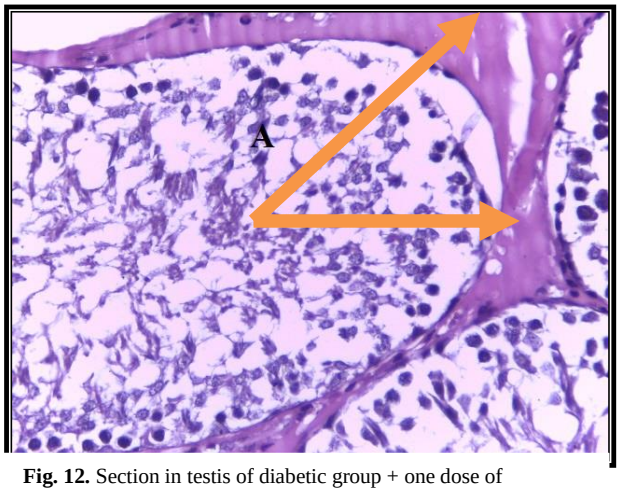

Fig. 12. Section in testis of diabetic group + one dose of Metformin exhibiting increasing thickness of connective tissue; (H&E; 100 X).

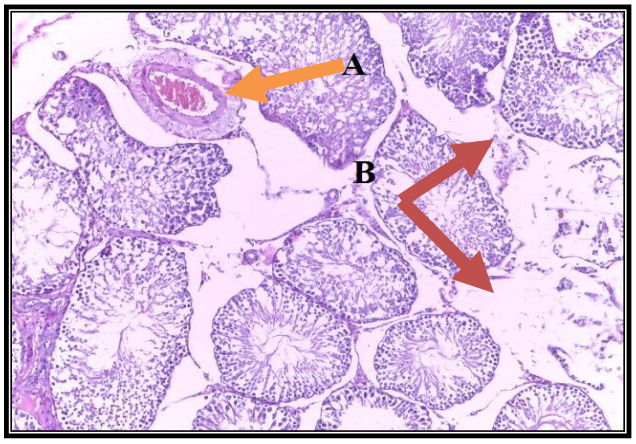

Fig. 13. Section in testis of diabetic group + one dose of metformin illustrating congestion (A); the destroyed interstitial tissue (B); (H&E; 100 X).

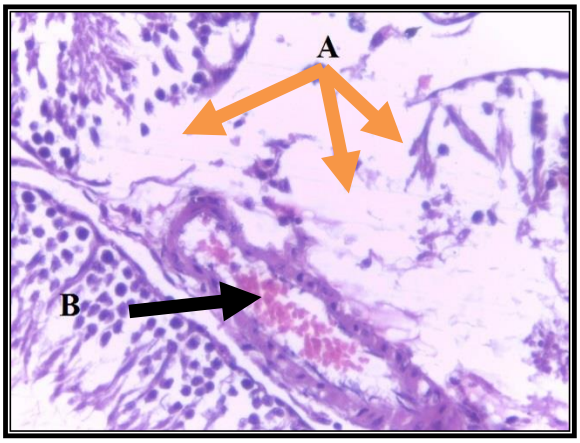

Fig. 14. Section in testis of diabetic group + two doses of metformin displaying the destroyed seminiferous tubules clearly (A) congestion of blood vessel (B); (H&E; 100 X).

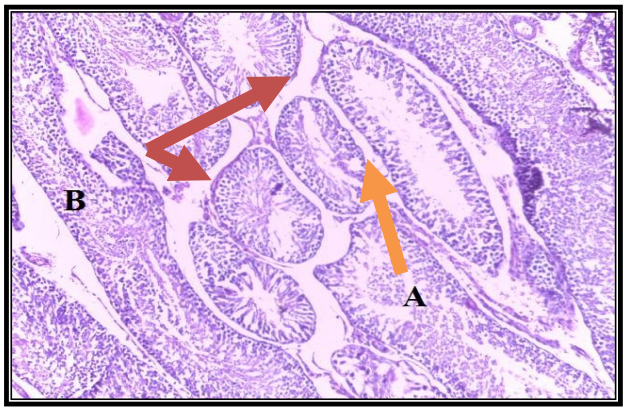

Fig. 15. Section in testis of diabetic group + two doses of metformin revealing the necrosis of spermatocytes (A); destroyed interstitial tissue (B); (H & E; 100 X).

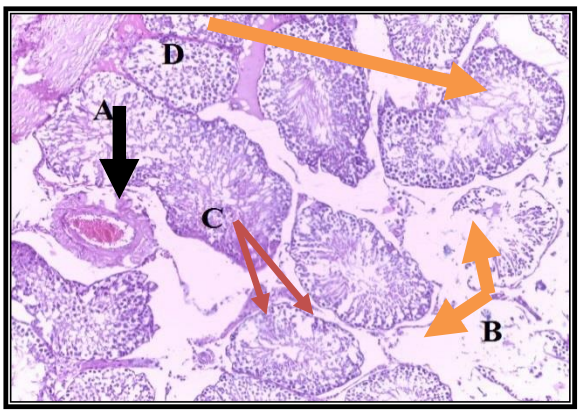

Fig. 16. Section in testis which most affected of diabetic group + two doses of metformin indicating congestion (A); destroyed interstitial tissue (B); necrosis (C); reduction of sperm (D); (H & E; 100 X).

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