Effects of Simvastatin and/or Metformin Administration on Lipid Profile and Reproductive Function in Adults Male Rats

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Abstract

Background : HMG-CoA reductase inhibitors, are large used lipid lowering medical that are effective in the functions of male reproductive. In addition, Metformin is affected on level of cholesterol that results in production of sperm. This study was designed to evaluate whether influence of simvastatin, metformin alone and combination in male rats. **Methods**: In this experiment 24 adult male rats were randomly divided into four groups(n=6) as following: control group (G1). 2nd group(G2)20 mg/kg simvastatin. 3rd group(G3)150mg/kg of metformin. 4th group(G4)150mg/kg metformin + 20m/kg simvastatin. The administration rout by oral gavage for 40 days. **Results**: There is a significant increase ($P \leq 0.05$) in blood parameters, HDL cholesterol, blood glucose, total protein and sperm abnormality in treated groups. Histopathological study of testes reveals degeneration, vacuolation of germinal epithelium, destruction the wall of some seminiferous tubules. Liver tissue shows dilated, congested sinusoid, vacuolation of hepatocytes and central vein congestion, on the other hands, kidney reveals a vacuolation of some epithelial lining of glomeruli and renal tubules, atrophy of some glomeruli and dilation of bowman`s capsules. **Conclusion**: Simvastatin have a better effect on lipid profile. On the other hands, have more effects on testicular function.

Key Words: *Metformin, Simvastatin, HMG-CoA reductase.*

Introduction

Statin affect cholesterol synthesis and thus may be contribute in inhibits steroidogenesis[(1)]. Due to their cholesterol-decreasing properties, this class of inhibitors might be expected to have adverse effects on reproduction by reducing the supply of circulating cholesterol which is required for steroidogenesis [(2)]. Nevertheless, HMG-CoA reductase inhibitors are considered teratogenic due to studies conducted with lovastatin [(3)], and sporadic testicular effects have been observed in dogs [(4,5)]. Metformin controlling blood glucose level. Metformin works by helping to restore the body's response to insulin. It decreases the amount of blood sugar that the liver produces and that the intestines or stomach absorb [(6)]. Metformin have a positive impact on sperm quality, this due to it is ability to reduce oxidative stress and lipid peroxidation, enhance 5'-AMP activated protein kinase activity, and restore the normal levels of pituitary-gonadal hormones [(7)].

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Materials and Methods

This study was conducted in College of Pharmacy and it's approved by the ethical committee in the College. In this study,24 adults male rats (335 -365 g) were used. Rats housed in plastic cages (2 rats /cage) under standard laboratory conditions (12/12 light/dark cycle, 22 ± 2 ˚C), they are allowed for pellet and tap water.

Experimental design

Animals are divided randomly into 4 equal groups (n=6). The control group dosed with D.W. for 40days, simvastatin group 20mg/kg simvastatin, metformin group 150mg/kg metformin, simvastatin (20mg/kg) and simvastatin + metformin group dosed 20mg/kg simvastatin and 150 mg /kg metformin. Blood was

collected from inferior vena cava, serum was stored in Eppendorf tubes at -20C°. Blood glucose was measured according[8], total cholesterol (TC)[9], Triglyceride (TG) [10], HDL cholesterol with total cholesterol[11]. VLDL according[12]. Sperm count according[13]. Individual sperm motility according[14]. The organs fixed in 10% formalin, stained with H&E and examined under light microscopy.

Statistical Analysis

The data were expressed as mean ±Standard deviation (SD), ANOVA analysis in our study. Least significant difference (LSD) was used to test the differences among means, indicated a significant (P<0.05), using computerized SPSS v3.

Results

1.Biochemical tests and Sperm analysis

Table(1) showed that was a significant increase (P≤0.05) in HB, PCV, RBCs and WBCs count of simvastatin group compared with control and other treated groups. While, it showed a significant decrease in PTL count of all treated groups compared with control group.

Table (2); the serum cholesterol, TG, and VLDL cholesterol level of sim, metf, and co-treated sim with metf groups were significantly lower than control group (P≤0.05). While, markedly increase level of HDL cholesterol was observed in sim rats compared with control and other treated rats. Also, serum level of LDL cholesterol remained elevated in the control and metf rats compared with sim and co-treated rats.

Effects of metformin and simvastatin on serum blood glucose and plasma total proteins in adults male rats was shown in table (3). Compared with met, and co-treated sim-metf grups the serum level of blood glucose were significantly higher in group sim (p≤0.05). On the other hand, the level of total proteins in co-treated group were significant higher than all groups are studies. However, simvastatin, metformin and co-treated rats showed a significant increase in abnormal sperm morphology compared with control.

Table (1) Effect of metformin, simvastatin and co-administration of metf. and simv. on blood parameters. (M±SD)(n=6)

Parameters groups	HB g/dl	RBC ×106cell/mm3	WBCs ×103cell/mm3	PCV %	PLT %
G1 cont.	13.25±0.6 d	6.06±0.1 d	11.51±0.2 b	37.53±1.9 C	443±25.8 a
G2sim	15.61±1.1 a	6.39±0.4 a	14.63±2.5 a	39.44±0.2 a	269±43.8 d
G3metf	14.26±0.8 c	6.10±0.2 c	9.96±1.1 d	36.46±1.5 d	410±15.2 b
G4sim,metf	14.68±06 b	6.37±0.2 b	11.31±0.6 c	38.71±3.5 b	363±25.8 c
LSD	0.41	0.02	0.20	0.72	24.81

The different letters refer to significant differences among groups at level of (p≤0.05).

Table (2) Effect of metformin, simvastatin and co-administration of metf. and sim. on lipid profile. (M±SD) (n=6)

Parameters groups	Chl. mg/dl	Tg. mg/dl	HDL mg/dl	VLDL mg/d	LDL mg/dl
G1 cont.	203.68±2.7 a	157.61±9.5 a	71.36±3.7 d	31.52±1.9 a	99.065±25.5 a
G2sim	176.06±7.4 d	78.38±8.0 d	185.01±5.5 a	15.73±1.6 d	66.06±7.9 c
G3metf	181.85±9.5 b	123.08±7.3 b	84.23±4.9 c	24.61±1.4 b	97.04±0.5 a
G4sim,metf	181.11±11.8 c	104.60±2.4 c	145.65±10.5 b	20.83±0.5 c	85.13±12.9 b
LSD	0.73	18.46	12.87	3.77	2.61

The different letters refer to significant differences among groups at level of (p≤0.05)

Table (3) Effect of metformin, simvastatin and co-administration of metf. and sim. on blood glucose and total proteins. (M±SD)(n=6)

Parameter groups	Blood glucose g/dl	Total proteins g/dl
G1cont.	177.10±28.0 a	14.23±1.3 d
G2 sim	184.06±8.2 a	17.61±1.9 b
G3met	117.33±37.3 b	16.75±0.6 c
G4sim,met	140.83±21.9 b	17.71±1.8 a
LSD	36.33	0.10

The different letters refer to significant differences among groups at level of (p≤0.05)

2.Histopathological study

1. The Testes

Testes of control rat include normal seminiferous tubules. Testes of rat treated with simvastatin(20mg/kg B.W), shows degeneration with vacuolation of germinal epithelium and irregularity of basement membrane. Few numbers of spermatogonia as well as necrotic spermatocytes in some seminiferous tubules. Testes of rats treated with metformin (150 mg/kg b.w) shows vacuolation of germinal epithelium. There few numbers of spermatogonia. Testes of rats treated with simvastatin(20mg/kg b.w)and metformin(150mg/kg b.w) shows disarrangement, sever sloughing of epithelium and destruction the wall of some seminiferous tubule. There is a lot of number of spermatids in seminiferous tubules.

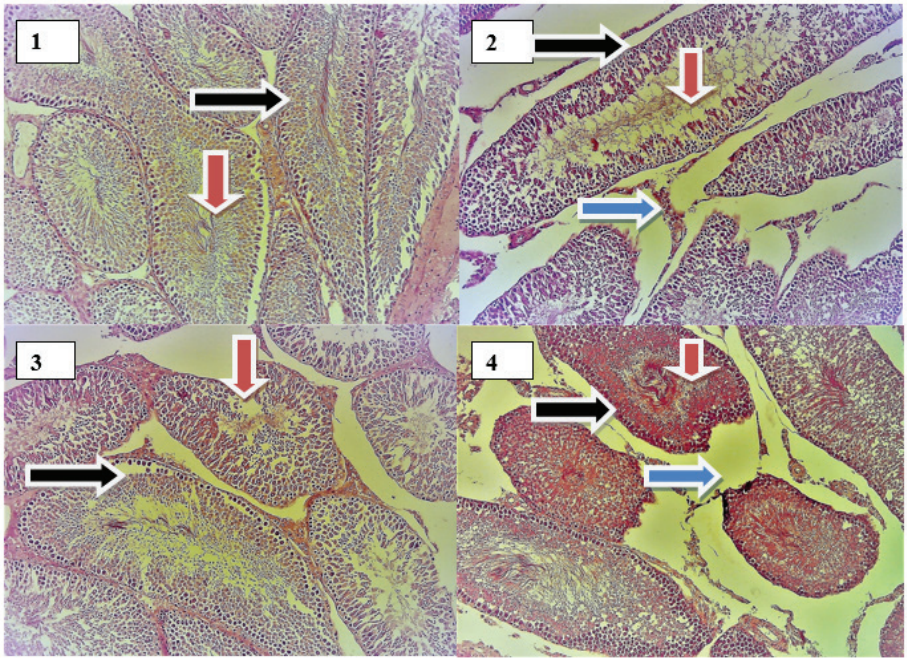


Figure (1): tests of control, normal structure (black arrow) full with sperm(red arrow). (2): tests of rat treated 20mg/kg simvastatin, degeneration in addition to vacuolation (red arrow) (3): tests of rat treated 150mg/kg metformin, vacuolation &degeneration(red arrow).(4):tests of rat treated simvastatin and metformin, thickens within semineforus tubules and vacuolization(red arrow). H&E, 200x.

2. The Liver

The liver tissue of control group has shown normal liver tissue. The cellular cords separated by sinusoids. The plate hepatic cells were separated by narrow, congested blood sinusiods with vacolation of hepatocytes(figure 2). Also there is vacuolization of hepatocytes, congested central vein, enlarged sinusoid(figure 2). Hepatic nuclei are enlarged pyknotic and light chromatinic stained, disappearance of the radiated hepatic architecture, massive vacuolation in hepatocyte, the central vein thick and narrow(figure 2).

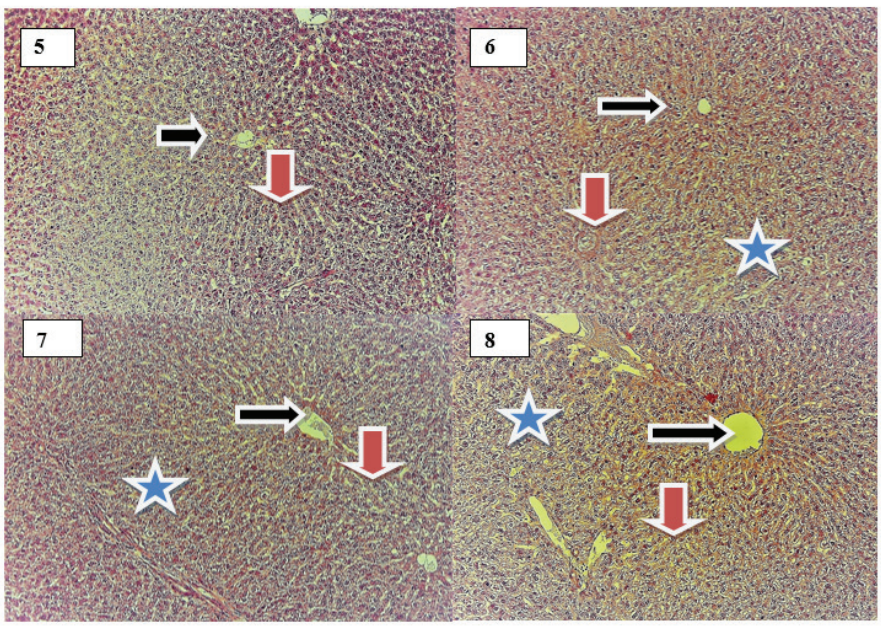
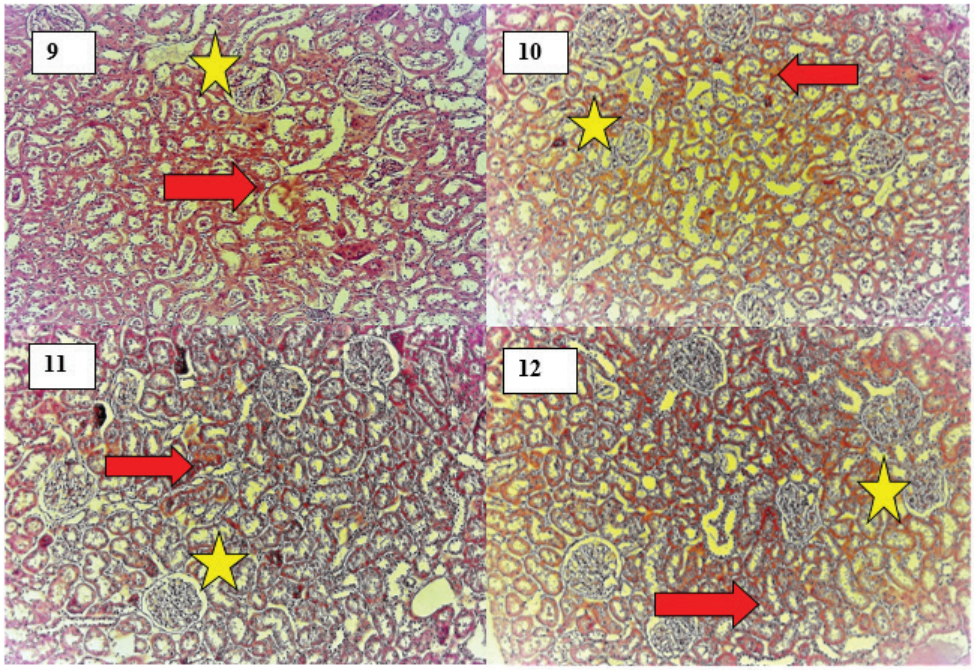


Figure (2): liver control rat (black arrow) and hepatocytes (red arrow).(6): liver of male rat exposed 20mg/kg B.W.of simvastatin, vacuolization (blue star), congested of sinusoid (red arrow).(7): liver of male rat exposed 150mg/kg B.W.of

metformin,vacuolization, congested central vein(black arrow),enlarged sinusoid (blue star).(8): liver of male rat exposed 20mg/kg B.W. of simvastatin and 150 mg/kg B.W of metformin, vacuolated hepatocyte. H&E, 200x.

3. The Kidney

The kidney of control shown normal renal tissue(figure 3). Kidney of rats exposed to 20mg/kg B.W simvastatin, **shows** necrosis of the epithelial lining of glomeruli and tubules and glomerular atrophy(figure 3). Kidney of rat exposed to 150mg/kg B.W. of metformin, **shows** enlargement of glomruli with dilatation of bowman's capsule and hemorrhage between tubules(figure 3). Kidney of rat exposed to 20mg/kg B.W of simvastatin and co-treated with 150mg/kg B.W. of metformin, **shows** atrophy of glomeruli with narrow of bowman's capsule and hemorrhage between tubules(figure 3).



Figure(3): kidney of control rat show normal glomeruli(star)and tubules(red arrow).(10): kidney of rat 20mg/kg B.W. of simvastatin, necrosis of the epithelial glomeruli, tubules(star), atrophy of glomeruli(red arrow). (11):kidney of rat 150mg/kg B.W.of metformin, enlargement of glomeruli, dilatation of bowman's capsule(star), hemorrhage between tubules(red arrow). (12): kidney of rat 20mg/kg B.W of simvastatin and 150mg/kg B.W. of metformin, glomerular atrophy , narrow of bowman's capsule (star), hemorrhage(red arrow). H&E, 200x.

Discussion

The continues increasing of glycosylated hemoglobin as product of diabetic related with glucose over all in blood is give form to changes of Hb structure, this factors could have contribute to increase in all blood cells. Therefore, hyperglycemic increases the blood cells count[(15)]. Hence, the altered in blood picture (RBCs, WBCs, PCV, and PLT) may presented in table(1)[(16)]. Another interesting finding of our study is that the effect of simvastatin, metformin & combination on lipid profile in which there is a significant decrease in lipid profile of all treated groups in comparison to control group while there is a significant increase in HDL in simvastatin group when compared with untreated rats as shown in table (2). Our result is in line with[(17)] who mentioned that there is a decrease in all lipid profile in mice treated with atorvastatin.

Several studies showed that rats treated with metformin cause a decrease in lipid profile, while the addition of natural honey to metformin significantly decrease all type of cholesterol and has a better effect in comparison to metformin alone in diabetic rats[(18,19)]. Our results are also in agreement with Wang etal[(20)], who observed that the level of TC, TG significantly decrease & the HDL cholesterol increase in diabetics rat treated with simvastatin drug. Bellia etal, mentioned

that random clinical studies of patient treated with simvastatin drug at dose 20mg/day has no effect on glucose level control for 4weeks of treatment, also found that glycemic control and HbA1c may worsen with increase duration of treatment till 1year with no change in insulin sensitivity[21,22].

Result in table(3)revels the increment in glucose level in rats treated with statin drug significantly in comparison to those treated with metformin &the combination of these two drugs, this result may be related to the effect of statin drug on islet of Langerhans which may effect on their ability to secret insulin or may be due to decrease the sensitivity of insulin which lead to increase insulin resistance and increase glucose level. This result is in agreement with[23,20] they both reported that simvastatin associated with an increase in serum glucose level and impaired glucose homeostasis in patients and rats respectively.

The present study showed an effect of simvastatin, metformin and their combination on sperm count, motility and normal morphology presented as a decrease in sperm characteristics, on the other hands, these drugs also increase the abnormality of sperm morphology in comparison to control group , this result is in agreement with[24] who mentioned that a decrease in testes weight, low sperm count, motility and abnormal morphology of semen in rat administered high dose atorvastatin.

Adaramoye and Lawal,[25]; Banihani,[26] they mentioned that administering of metformin in rat may have a positive effect on sperm characteristics especially in diabetic rat and at dose 30mg/kg per day for 6weeks. While administering of metformin for 3 weeks at the same above dose in rat associated with a decrease in semen count and motility by 33%[27]. Our results corresponding with histopathological study of testes tissue associated with degeneration, vacuolation of epithelium, sloughing in germinal epithelium and spermatocytes necrosis in some of seminiferous tubules, this results is in line with(28,29) they reported that rat treated with rousovastatin and atorvastatin respectively cause changes in seminiferous tubules, death of some germinal epithelium and edema in interstitium.

Drugs exposed to metabolism by liver and kidney tissue, therefore histopathological study of these organs associated changes including dilation of liver sinusoid, vacuolation of hepatocytes and congestion of hepatic sinusoids. Kidney associated with excretion of drugs metabolites therefore in some field showed atrophy of some glomeruli, dilation of bowman`s capsules and degeneration of some glomerular cells. These changes occur as an adaptation of these organ to accommodate the continuous administration of these drug, therefore considered as reversible changes and the organs can back to normal state if the cause annotate[30].

Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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References

1. Marek M, Eliza K, Pawel J, Malgozata SL, Anna T, Alicija F. Treatment with statines and testosterone levels in me. Endo Polska.2014; 65(6):464-468.
2. Dobs AS, Sarma PS, Schteingart D. Long-term endocrine function in hypercholesterolemic patients treated with pravastatin, a new 3-hydroxy-3-methylglutaryl coenzymeA reductase inhibitor. Metabolism.1993;42,1146-1152.
3. Minsker DH, MacDonald JS, Robertson RT, Bokelman DL. Mevalonate supplementation in pregnant rats suppresses the teratogenicity of mevinolinic acid, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Teratology.1983;28,449-456.
4. MacDonald JS, Gerson RJ, Kornbrust DJ, Kloss MW, Prahalada S, Berry PH, Alberts AW, Bokelman DL. Preclinical evaluation of lovastatin. Am. J.Cardio.1988;62,16J-27J.
5. Gerson RJ, MacDonald JS, Alberts AW, Kornbrust DJ, Majka JA, Stubbs RJ, Bokelman DL. Animal safety and toxicology of simvastatin and related hydroxy-methylglutaryl-coenzyme A reductase inhibitors. Am. J.Med.1989;87(4A),28S-38S.
6. Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: An update. Ann Intern Med.2002; 137:25–33.

7. Banihani SA. Effect of metformin on semen quality. Brit J of Pharma Sci.2016;52(4) :591-595.
8. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of Clin Bio.1969;6:24-27.
9. Allian CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin. Chem.1974;20(4):470-475.
10. Fossati P, Prencipe L. Serum triglyceride determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem.1982;28:2077-2080.
11. Tietz NW.Textbook of clinical chemistry,3rd ED. C.A. Burtis, E.R. Ashood, W.B. Saunders 1982;819-86.
12. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem.1972;18:499-502.
13. Evan G, Maxwell WMC. Salamones artificial insemination of sheep and goat . Butterwoths .Sydney, Australia.1987;1-94.
14. Chemineau P, Cagnie Y, Gue'rin Y, Orgeur P, Vallet JC. Training manual on artificial insemination in sheep and goat. FAO, Rome,1991.
15. Alamri BN, Bahabri A, Aldereihim AA, Alabduljabbar M, etal. Hyperglycemia effect on red blood cells indices. Euro rev for med and pharma sci.2019;23(5),2139-2150.
16. Abboud SH, Abbas MK, MehdyZaini SS, Naqi MT. The potential effects of short course of Statins on some platelets parameters in rats. AL-Qadi Med J.2014;10(18):94 -97.
17. Bustan AA, Jawad AM. The Effect of Two Types of Statins (Rosuvastatin and Atorvastatin) on the Fertility of Male and Female Mice. Brit J Med &Med Res.2017;19(12):1-11.
18. Erejuwa OO, Sulaiman SA, Ab-Wahab MS, etal. Glibenclamide orMetformin Combined with Honey Improves Glycemic Control in Streptozotocin-Induced Diabetic Rats. Int. J.Biol. Sci.2011;7:244-252.
19. Nasrolahi O, Heidari R, Rahmani F, Farokhi F. Effect of natural honey from Ilam and metformin for improving glycemic control in streptozotocin-induced diabetic rats. Avicenna J of Phytomed.2012;2(4):212-221.
20. Wang L, Duan G, Lu Y, Pang S, Huang X, Jiang Q, Dang N. The Effect of Simvastatin on Glucose Homeostasis in Streptozotocin Induced Type 2 Diabetic Rats. J Diab Res,ID 274986: 2013;1-5.
21. Bellia A, Rizza S, Galli A, Fabiano R, Donadel G, Lombardo MF. Early vascular and metabolic effects of rosuvastatin compared with simvastatin in patients with type 2 diabetes. Atherosclerosis.2010;210(1):199-201.
22. Bellia A, Rizza S, Lombardo MF, etal. Deterioration of glucose homeostasis in type 2 diabetic patients one year after beginning of statins therapy. Atherosclerosis.2012;223(1):197-203.
23. Toth PP, Catapano AL, Farnier M, Foody J, Tomassini JE, Jensen E. Effect on Fasting Serum Glucose Levels of Adding Ezetimibe to Statins in Patients With Nondiabetic Hypercholesterolemia. Am J Cardio.2016;118:1812-1820.
24. Klinefelter G, Laskey J, Amann R. Statin drugs markedly inhibit testosterone production by rat Leydig cells in vitro: Implications for men. Repro Toxi.2014;45:52-8.
25. Adaramoye OA, Lawal SO. Effect of kolaviron, a biflavonoid complex from Garcinia kola seeds, on the antioxidant, hormonal and spermatogenic indices of diabetic male rats. Andro.2014;46(8):878-886.
26. Banihani SA. Effect of metformin on semen quality. Bra J of Phar Sci.2016;52(4): 591–594.
27. Adaramoye O, Akanni O, Adesanoye O, Labopopoola O, Olaremi O. Evaluation of toxic effects of metformin hydrochloride and glibenclamide on some organs of male rats. Niger.J. Physiol. Sci.2012;27(2):137-144.
28. Leite GAA, de Lima Rosa J, Sanabria M, Cavariani MM, etal. Delayed reproductive development in pubertal male rats exposed to the hypolipemiant agent rosuvastatin since prepuberty. Repro Toxic.2014;44:93-103.
29. Das SS, Indira M. Combined effects of atorvastatin and nicotine on testicular expression of steroidogenic enzymes in experimental rats. Inter J Phar Sci and Res.2015;6(6):2511.
30. Khadhair AA, Majeed SK. Toxopathological and biochemical study of multiple doses digoxin in sprague dawley rats. Bas. J. Vet. Res.2018;17(1):136-146.