The positive effect of metformin and metronidazole on the diabetic rats experimentally

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Abstract

Diabetes mellitus is considering most serious disease of endocrine, it affecting large number of people in worldwide. This study was performed to insured the effect of combination of metformin and metronidazole on disorder in metabolic that concomitant with injection of alloxan IP induced diabetes in rats. In this study forty male rats: divided into four groups including control group G1, alloxn group (150 mg/kg single dose) G2, alloxn group orally treated with metformin (150mg/kg/BW) G3, and alloxn group orally treated with metformin (150mg/kg/BW) and metronidazole (500mg/kg/BW) G4. The experiment last for 14 days. The result shows marked increase in MDA, glucose and also in liver enzymes, lipid profiles and even observed disorder in kidney function test by increased in the creatinine, urea, and uric acid levels. In addition to study the histological changes in diabetic liver of rats characterized by kupffer cells activation, dilation and congestion of central vein and hepatic sinusoids. Administration of metformin and combination of metformin and metronidazole, result in significant reduction return abnormal parameters to the normal values. Also correct the abnormal histological changes of liver.

Keywords: alloxan, metformin, metronidazole, rats.

الخلاصة

يعتبر مرض السكري اخطر امراض الغدد الصم, ويوثر على عدد كبير من الناس في جميع انحاء العالم, وقد اجريت هذه الدراسه لتوثيق تاثير مزيج من المتفورمين والميترونيدازول على اضظراب التمثيل الغذائي في الجرذان المحدثة السكر بعد حقنها بالالوكسان. في هذه الدراسه

استخدمت اربعون من ذكور الجرذان: تقسم الجرذان الى اربع مجاميع, المجموعه الاولى مجموعة سيطره, المجموعه الثانيه تحقن بجرعه واحده من بالالوكسان داخل البريتون (150 ملغم/كغم من وزن الجسم), المجموعه الثالثه تعطى الميتفورمين فمويا (150 ملغم/كغم من وزن الجسم), المجموعه الرابعه تعطى الميتفورمين فمويا (150 ملغم/كغم من وزن الجسم) مع الميترونيدازول (500 ملغم/كغم من وزن الجسم) فمويا. التجربه استمرت 14 يوم. اظهرت النتائج زياده ملحوضه في مستويات المالونديهايد, السكر, انزيمات الكبد, مستوى الدهون, ايضا لوحظ اضطراب في وظائف الكليه عن طريق زياده مستويات الكرياتنين, اليوريا وحامض اليورك. بالاضافه الى دراسة التغييرات النسيجيه في كبد الجرذان المصابه بالسكر متمثله تنشيط خلايا كوبفر, توسع واحتقان في الوريد الكبدي, وفي الجيوب الكبديه. اضافة الميتفورمين واضافة الميتفورمين مع الميترونيدازول ادى الى انخفاضا معنويا في النسب الغير طبيعيه, و ايضا اظهرت النتائج قدرت الميتفورمين والميترونيدازول على تحسين التغييرات النسيجيه في الكبد

Introduction

The term diabetes mellitus (DM) is considered as disorder in metabolism of fat, protein carbohydrate, causing increase in blood sugar resulting from defects in insulin secretion, insulin sensitivity or both (WHO, 1999; Baynes, 1999). Also the effect of DM result in dysfunction of many organs. It characteristic by polyuria, thirst, blurring of vision and loss of body weight (WHO, 1999). Also this disorder is associated with profound alterations in lipid profile and with an increased risk of coronary heart disease (Saravanan and Pari, 2006).

Metformin is an only member of biguanide, and generally it is the first-choice oral drugs in the treatment of type 2 DM due to it has potent antihyperglycemic effect, important effect on body weight, and not expensive. Using of other antidiabetic drugs with metformin will produce synergism antidiabetic effect (Standl and Schnell, 2012).

Metronidazole is one of those chemotherapeutic agents that bean have used in treatment of ailments caused by anaerobic bacteria and certain anaerobic protozoa, such as Trichomonas vaginalis, Entamoeba histolytical, and Giardia lamblia (Dean *et al*., 1989). Metronidazole has been reported to induce a reversible bone marrow depression and cytotoxic effects on male mice fertility (El-Nahas and El-Ashmawy, 2004). Administration of Metronidazole with high doses caused harmful effects on some organs and affects males' fertility in rats (Schwartz *et al*., 1979; Mudry *et al*., 2007). With using of Metronidazole, several

neurologic side effects may occur, including headache peripheral neuropathy, and encephalopathy (Hobson *et al*., 2006), in addition to gastrointestinal upset, metallic taste and rarely pancreatitis (Nigwekar and Casey, 2004).

The aim of study to investigate the effect of metronidazole on the antidiabetic and antihyperlipidemic effect of metformin in induced diabetes rats, as well as on some tissue markers of oxidative stress (malondialdehyde).

Materials and Methods

The experiment was conducted at the animal house of the Veterinary Medicine College-University of Basrah, were 40 male rats (*Rattus norvegicus*) sexually mature of 12 weeks age and 175-200 gram weight were used. These animals were housed in standard cages with controlled temperature (20 – 25oC) and 12 hours light / dark cycle with free access to water and standard pellets diet. One week after acclimatization in the laboratory where experiment were performed. The animals were divided randomly into 4 equal groups (10 rats in each group) and treated for 14 days as following: Control group G1: In which rats were treated with normal saline intraperitoneally injected (0.5 ml/day). Alloxan-induced diabetes group G2: Diabetes was induced by a single intraperitoneal injection of 150 mg/kg monohydrated alloxan (Sigma, St. Louis, MO, USA) dissolved in sterile 0.9% saline. Metformin group G3: Diabetes was induced and rats were treated with Metformine/orally/was administered at 150 mg/kg/BW/day/ suspended in physiological saline by gavage (Majithiya and Balaraman, 2006). Metformin and Metronidazole group (M+M group) G4: (Diabetes was induced and rats were treated with Metformin/orally at (150 mg/kg/BW/day) (Majithiya and Balaraman, 2006), and treated with Metronidazole/orally at (500 mg/kg/BW/day) suspended in physiological saline by gavage.

After overnight fast, the animals were sacrificed on the 15th day under mild chloroform anesthesia and blood was obtained via cardiac puncture. Blood was collected from the heart by 10 ml disposable syringe of 22G needles (Parasuraman, *et al*., 2010). One ml of blood was transferred into EDTA tube for hematological investigations, the remaining volume of blood transferred into plain tube and centrifuged at 3000 rpm for 15 minutes to obtain the serum which then transferred into

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Eppendorf tubes and stored at -20ºC untill used for measurement of different parameters.

Results

In the diabetic rats there are abnormalities in many parameters, in table (1): there are a significant increasing (P<0.05) in the MDA and glucose in diabetic group compared with the control group, while in metformin group the increasing in the MDA and glucose levels were reduced significantly (P<0.05) compared with those in control group and diabetic group. In M+M group (metformin and metronidazole group, there are more correction of these parameters with metronidazole present.

Table (1): The ameliorative effects of metformin and metronidazole on alloxan treated rats on MDA and glucose M+SE

Group	MDA nmol/mL	Glucose mg/dL
G1	85.62 ± 2.08 a	88.3 ± 0.83 a
G2	170.5 ± 1.16 b	166.9 ± 0.84 b
G3	140.43 ± 1.41 c	101.7 ± 1.36 c
G4	100.2 ± 1.03 d	99.5 ± 0.41 c
LSD	17.1	2.27

The obtained results in Table (2) revealed that the administration of alloxan was associated with significant increase (P< 0.05) in serum ALT, AST and ALP levels when compared with the control group. The elevation in serum level of the enzymes was nonsignifecantly reduced from those in control group by administration of Metformin. While administration metformin plus metronidazole resulted in significant reduction (P< 0.05) in serum ALT and AST when compared with alloxan and metformin group.

Table (2): The ameliorative effects of metformin and metronidazole on alloxan treated rats on liver enzymes M+SE

Group	ALT(U/L)	AST(U/L)	ALP (IU/L)
G1	9.3 ± 0.7 a	17.4 ± 0.09 a	50.5 ± 0.24 a

G2	20.5 ± 0.14 b	43.8 ± 0.12 b	90.9 ± 0.27 b
G3	13.4 ± 0.11 c	22.5 ± 0.02 c	66.8 ± 0.18 c
G4	10.7 ± 0.10 a	19.5 ± 0.03 a	54.9 ± 0.14 d
LSD	1.39	2.12	4.38

The results in Table (3) revealed that the administration to alloxan cause significant increased (P<0.05) in TC, TG, LDL and VLDL and significant decreased in HDL values compared with the control group. Also administration of metformin led to decline significantly(P<0.05) in TG and decreasing significantly in TC, LDL and VLDL values compared with alloxan treated group, but TC, TG, LDL still higher than those of control value. While HDL shows decreased insignificantly compared with control group. Administration of M+M in four group result in return the parameters nearly to normal value.

Table (3): The ameliorative effects of metformin and metronidazole on alloxan treated rats on lipid profiles M+SE

Group	TC mg/Dl	TG mg/dL	HDL mg/dL	VLDL mg/dL	LDL mg/dL
G1	55.83±1.84 a	73.76±0.5 a	51.62±0.13 a	18.2±0.76 a	45.7±0.14 a
G2	82.82±4.33 b	103.52±0.43 b	33.62±0.22 b	28.24±0.85 b	78.38±0.15 b
G3	67.36±4.8 c	78.78±0.21 c	48.7±0.17 c	15.7±0.12 c	48.9±0.04 c
G4	55.15±2.42 a	75.64±0.43 d	51.14±0.35 d	19.8±0.07 d	47.18±0.14 d
LSD	11.97	1.88	2.41	0.98	1.47

Table (4) illustrated the effect of metformin and metronidazole on serum level of creatinine, urea and uricacid in rats given alloxan and compared with control group value. Injection of alloxan caused a significant increment (P<0.05) in serum level of creatinine, urea and in uric acid values. Administration of metformin led to decline significantly (P<0.05) in serum level of creatinine, urea and in uric acid values value compared with alloxan treated group, and it reached to normal values compared with the control value, while in metronidazole group these parameters are return to the normal values when compared with control group.

Table 4: The ameliorative effects of metformin and metronidazole on alloxan treated rats on serum level of creatinine, urea, uric acid M ± SE

Group	Creatinine	Urea mg/dL	Uric acid mg/Dl
G1	0.26±0.002b	32.34± 0.27a	3. 1±0.02a
G2	0.71±0.004a	37.9 ± 0.16b	3.84 ±0.01b
G3	0.41±0.004b	35.02 ±0.08c	3.32 ±0.02c
G4	0.27±0.006a	33.88 ±0.14a	3.4±0.02a
LSD	0.014	1.14	0.1

Histopathological changes in the liver (1,2,3,4)

Normal hepatic structure is shown from liver of control rats. In which, normal hepatocytes are arranged in cords radiating from the central hepatic vein and separated by normally looking hepatic sinusoids (Fig: 1). Whereas in Fig: 2, the liver of rats in alloxan group showed Kupffer cells activation, dilation and congestion of central vein and hepatic sinusoids that due to alloxan administration, while diabetic control group showed high level of cellular abnormalities including necrosis, cellular and vascular degeneration, vascular congestion, hyperplasia of the hepatocytes and vacuolation, these disorders is treated with metformin administration (Fig: 3). These effects of metformin is supported with administration of metronidazole (Fig: 4)
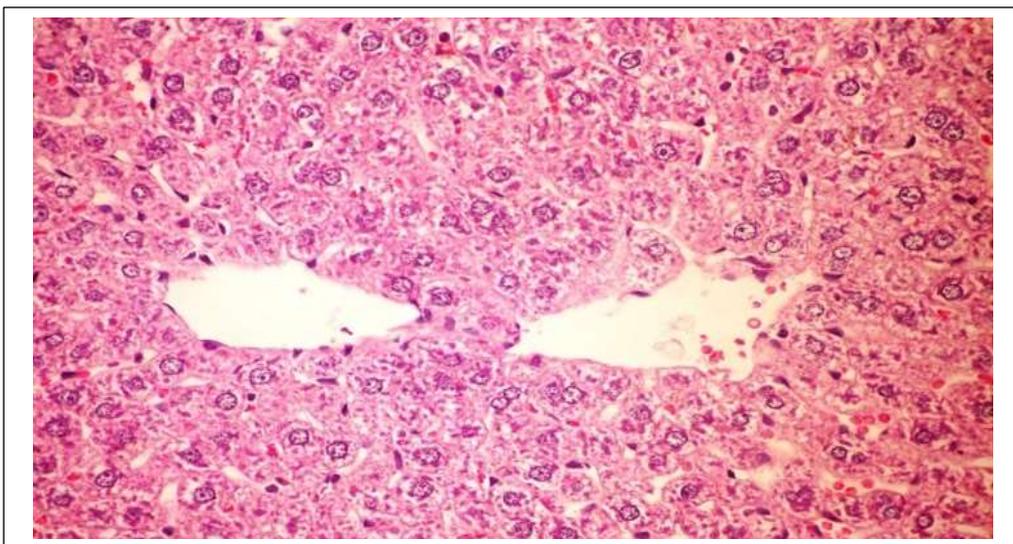
Figure 1: Liver of normal control rat showing the normal histological structure of central vein (star), and hepatocyte (black arrow) (H&E x 400)
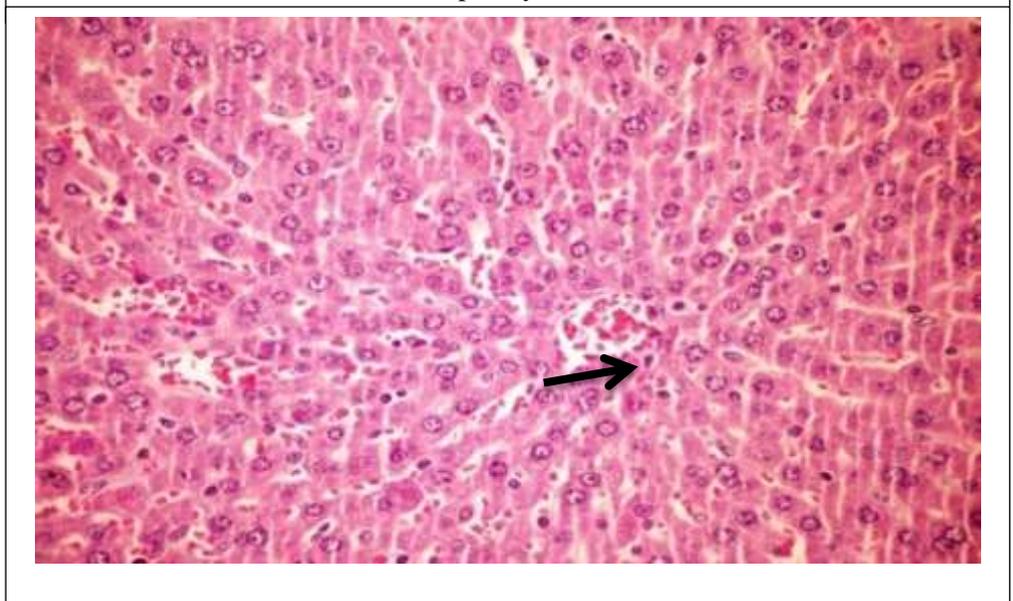

Figure 2: Liver of diabetic rat showing Kupffer cells activation, dilation and congestion of central vein and hepatic sinusoids in black arrow (H&E x
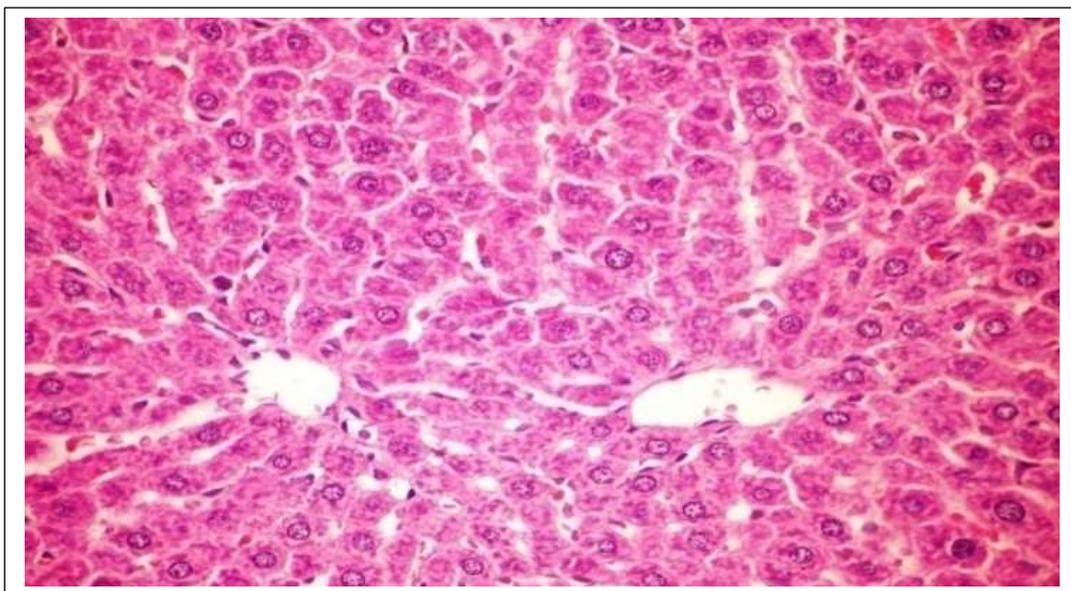
Figure 3: Liver section of diabetic rats treated with metformin alone showing nearly normal structures (H&E x 400)

Figure 4: Liver section of diabetic rats treated with metformin + metronidazole, showing nearly normal structures (H&E x 400)

Discussion

Diabetes mellitus is a chronic metabolic disorder involving carbohydrate, proteins and fat characterized by hyperglycemia and insufficiency secretion or action of endogenous insulin (Barar, 2002).

In diabetes mellitus, oxidative stress seems mainly to be due to increased production of free radicals and/or a sharp reduction of antioxidant defenses (Ahmed, 2005). Alloxan is one of the usual substances used for the induction of diabetes mellitus apart. It has a destructive effect on the beta-cells of the pancreas (Prince and Menon, 2000; Jelodar *et al*., 2003; Gwarzo *et al*., 2010), and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide with simultaneous massive increase in cystolic calcium concentration, which causes rapid destruction of pancreatic beta-cells of Islets of Langerhans thereby inducing hyperglycemia (Grover *et al.,* 2000; Szudelski, 2001). Insulin deficiency leads to various metabolic alterations in the animals such as increased blood glucose, total cholesterol, alkaline phophatase and transaminases (Begum and Shanmudnaram, 1978). Also the destruction of pancreatic beta cells is by alloxan cause release or increase level of Malondialdehyde (MDA) which is a degradative product of peroxidation of polyunsaturated fatty acids (PUPA) in the cells membrane. Presence of higher MDA in the serum is an indication of induced lipid peroxidation and of oxidative stress of which has been reported as one of the underlying cause of diabetes mellitus (Akinosun and Bolajoko, 2007). Liver enzymes such as AST and ALP are marker enzymes for liver function and integrity (Adaramoye, 2008)**.** In this study, increased activities of AST and ALT were observed in the diabetic animals. Hence it indicates that diabetes may induce hepatic dysfunction in rat. The increase in the level of these enzymes in diabetes may be as a result of leakage from the tissues and migration into the bloodstream (Chaudary *et al*., 1993). Diabetic patients may have damage to various tissues. The damage can increase serum level of ALT and AST. Levels of ALT may rise sharply if there is injury to hepatocytes, cardiac muscle, striated muscle, pancreas, kidney and erythrocytes. Serum level of AST wills increases when hepatocytes, cardiac muscle, striated muscle, and pancreas have damage (Keeffe and Friedman 2004). Excess of fatty acids in plasma produced by the alloxan-induced diabetes promotes the liver conversion of some fatty acids into triacylglycerol, phospholipids and

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cholesterol which may be discharged into the blood as lipoproteins (Bopanna *et al*., 1997). The main function of the kidneys is to excrete the waste products of metabolism and to regulate the body concentration of water and salt. Significant increase of total urea and creatinine levels indicated impaired renal function of diabetic rats which led to a negative nitrogen balance, enhanced proteolysis and lowered protein synthesis (Sulaiman *et al*., 2012)

Treatment of diabetic rats with metformin for 14 days showed a decrease in the elevated plasma MDA level which might indicate that metformin has a radical scavenging activity (Murad *et al*., 2015), and has protective effect against the hepatotoxicity produced by alloxan diabetes by decreased blood glucose levels, serum ALT and AST activities, total lipid. (Yanardag *et al*., 2005). Metformin is an oral hypoglycaemic agent that exhibits an antihpyerglycemic effect devoid of insulin release (Stumvoll *et al*., 1995). Metformin exerts its major effect through inhibition of hepatic glucose production; enhanced glucose disposal has also been described. Some early studies suggested that metformin exerts its insulin-sensitising effect (Bailey and Puah, 1986) and/or promotes glucose transport independently of the insulin receptor-mediated proximal signalling pathway in skeletal muscle (Hundal *et al*., 1992; Zhou *et al*., 2001; Turban *et al*., 2012). Also metronidazole were used in this study which is an antibiotic used in treatment of many anaerobic bacterial and protozal infection. It is rapidly absorbed from the digestive system (Turgut and Özyazici, 2004).

In this study, concomitant administration of metformin with metronidazole to diabetic result in improvement in abnormal parameters. Metronidazole may increase in the absorption rate constant, peak plasma concentration of metformin, increased the bioavailability and decrease in elimination rate constant of metformin. This potentiated effect of metronidazole to metformin is agreement with the report of the study carried out by Bakare-Odunola *et al*., (2001), also Abdullahi, 2015 reported the potentiation effects on metformin was observed with concomitant administration of a single dose of 1 g metformin with 400 mg of metronidazole tablets and 1 g ampiclox capsules by increasing the peak plasma concentration of metformin.

Also alloxan caused congestion of central vein of liver with marked expantion of sinusoidal spaces, pyknosis of nuclei of hepatocytes (Bollen

et al., 1998). After administration of metformin, there is a histopathological assessment of liver tissues confirmed the membrane stabilizing and cytoprotective potential against the toxic manifestations induced by alloxan (Kanchan *et al*., 2012), which more improved with concomitant administration with metronidazole.

Conclusion includes protective effect of metformin against DM in rats induced by alloxan and potentiates this effect with concomitant administration with metronidazole.

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