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THE MODULATORY EFFECT OF ANTIDIABETIC DRUGS ON THYROID FUNCTION IN DIABETIC RATS

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ABSTRACT

The effects of 30 days treatment with chlorpropamide (5 mg/kg/day), glipizide (2.5 mg/kg/day), and metformin (350 mg/kg/day), on blood glucose level, serum fructosamine, glycosylated haemoglobin (HbA_1), triiodothyronine (T_3) and thyroxine (T_4) in diabetic rats were investigated. Animals were randomly assigned into three equal groups, each group received a single daily dose of one of the previously mentioned antidiabetic drugs. The parameters of interest are demonstrated before, ten and thirty days after drugs' administration. In the present study, all the used drugs significantly reduced the blood glucose level after 10 and 30 days. Both fructosamine and HbA_1 are significantly elevated after 30 days of chlorpropamide and glipizide administration, while the two paramaters were non-significantly changed in the group received metformin. Triiodothyronin was significantly decreased andthi was increased in all groups after 30 days of treatment. It could be concluded that, both chlorpropamide, glipizide and metformin decreased the elevated blood glucose level in diabetic rats. The change in fructosamine and glycolated haemoglobin was not indicative to the change in blood glucose level induced by glipizide, chlorpropamaide and metformin as the 30 days of study did not cover the required period needed for such correlation to occur. Triiodothyronine and thyroxin levels were decreased and increased respectively in diabetic rats treated by chlorpropamaide or glipizide indicating a modulatory action of such antidiabetic agents on the thyroid function. Metformin lowered T_3 but showed a limited effect on T_4 level.

INTRODUCTION

Glibenclamide, gliclazide and glipizide represent the second generation sulfonylureas[1]. Sulfouylureas exert their hypoglycemic effect by both pancreatic and extrapancreatic action. The pancreatic action includes stimulation of insulin release from ß-cells[2], while the extrapancreatic action includes potentiation of the action of insulin in liver and peripheral tissues[3,4], increase the concentration of insulin receptors in peripheral tissues[5], suppression of the hepatic gluconeogenesis[6] and reduction of hepatic insulin clearance[7].

Metformin, an antidiabetic biguanide drug has no effect on ß-cells and insulin production, but acts mainly by extrapancreatic mechanism[8].

Thyroid hormones have important role in body metabolism especially those of carbohydrates, lipids and proteins. Thyroid hormones accelerate utilization of carbohydrates[9], stimulate synthesis, mobilization and degradation of lipids[10] and increase protein synthesis[11]. But, large doses of T_4 inhibit protein synthesis and elevate amino acid level[12]. Insulin secretion is regulated, in part, by adrenergic mechanisms which are thyroid hormone dependent[11]. In diabetic patients there is abnormal thyroid function[13], usually serum T_3 level is reduced[14], T_4 level may be normal[15] or elevated[16].

The present work has been undertaken in an attempt to investigate the effect of some oral hypoglycemic drugs, chlorpropamaide, glipizide and metformin on blood glucose, serum serum fructosamine, HbA_1, T_3 and T_4 levels in diabetic rats. As thyroid hormone levels are changed in dabetic state, our study is designedto investigate the possible modification which may occur in the changes of thyroid hormone levels by the use of oral hypoglycemic drugs.

MATERIALS AND METHODS

Animals: Thirty adult male albino rats weighing 200-250 g were used in the present study. Diabetes was induced by subcutaneous injection of alloxan 150 mg/kg[17]. Animals with blood sugar level of 300 mg/100 ml or more are considered diabetic, eighteen rats out of the 30 were considered diabetic.

Exprerimentel design: The rats were randomly assigned to three main groups (each of 6 rats) and were given the individual antidiatetic drug orally via stomach tube in the following manner:

Group I: received chlorpropamide (5 mg/kg/day)[18] suspended in 1% tween 80 solution.

Group II: received glipizide (2.5 mg/kg/day)[19], suspended in 1% tween 80 solution.

Group III: received metformin (350 mg/kg/day)[20], dissolved in distilled water.

Blood samples were taken from the orbital sinus of the eye via glass capillaries, before treatment, ten and thirty days after drug administration. Blood samples were drawn into two tubes one for the blood to be centerifuged for determination of serum glucose, fructosamine, T_3 and T_4, in the other tube a small

volume of blood (about 1 ml) was drawn on EDTA for estimation of plasma HbA_1.

Methods: 1- Blood glucose level was determined by enzymatic colorimetric technique as described by Trinder[21] using glucose kit from Stanbio Laboratory Inc.-San Antonio-Texas-U.S.A..

2- Fructosamine was determined by nitroblue tetrazolium (NBT)[22,23] using fructosamine kit from QCA-Amposta-Tarrgona-Espain.

3- HbA_1 was determined by quantitative colorimetric method described by Trivelli et al.[24] using glycolated haemoglobin kit from Stanbio Laboratory Inc.-San Antonio-Texas-U. S. A.

4- Triiodothyronine (T_3) was determined by enzyme immunoassay (EIA) test[25] using T_3 EIA kit from Diatech Diagnostic Inc. Boston. . U.S.A.

5- Finally T_4 was determined by EIA test as described by Diatech Diagnostic Inc. laboratory data using T_4 EIA kit from Diatech Diagnostic Inc-Boston-U.S.A.

Statistical analysis:

All values are expressed as mean ± standard error of the mean. Statistical analysis was done using Student's-t- test for paired observations.[26]

RESULTS

1- Effect on blood glucose level:

The average blood glucose level of diabetic rats was (304.29 ± 15.93) mg/dl. Administration of chlorpropamide, glipizide and metformin significantly reduced blood glucose level to 72.2%, 71.9% and 69.66% of the control value after 10 days respectively and to 47.22%, 47.95% and 59.93% of the control value after 30 days respectively, Fig. (1).

2- Effect on fructosamine level:

Administration of chlorpropamide or glipizide significantly increased fructosamine level to 122.31% and 145.15% of th control value respectively only after 30 days. Administration of metformin produced non-significant reduction. The results are shown in Table (1).

3- Effect on glycolated haemoglobin level:

The level of HbA_1 was significantly increased to 130.97% of the control value after 30 days of treatment with chlorpropamide and to 109.8% of the control value after 10 and 30 days of treatment with glipizide, respectively. Administration of metformin produced non-significant reduction, Table (2).

4- Effect on T_3 level:

Administration of chlorpropamide significantly reduced serum T_3 level only after 30 days to 60.32% of the control value. Administration of glipizide or metformin significantly reduced T_3 level to 55.39% and 77.11% of the control value respectively after 10 days and to 53.85% and 61.45% of the control value respectively after 30 days. The results are shown in Table (3).

5- Effect on T_4 level:

Administration of chlorpropamide significantly increased serum T_4 level to 137.6% and 133.89% of the control value after 10 and 30 days respectively. Administration of glipizide significantly increased T_4 level to 170.74% and 154.21% of the control value after 10 and 30 days respectively. Administration of metformin also significantly increased T_4 level to 124.72% and 114.75% of the control value after 10 and 30 days respectively. The results are shown in Table (4).

DISCUSSION

The present data reveal that, both chlorpropamide and glipizide significantly lower the elevated blood glucose level after 10 and 30 days. These findings are in agreement with the previous investigations[27,28] and may be attributed to the stimulatory effect of sulfonylureas on pancreatic ß-cells to release insulin[1,29,30]. Sulfonylureas exert their hypoglycemic action due to extrapancreatic effects on liver and peripheral tissues.Also, Sulfonylureas enhance peripheral tissues sensitivity to insulin[31], by increasing the number of insulin receptors per cell[32], or by post-receptor effects[33,34]. Hatao et al.,[34] suggested that chlorpropamide stimulates synthesis of fructose-2,6-biphosphate (F-2,6-P_2) in the hepatocyets followed by enhancement of hepatic glycolysis and inhibition of liver gluconeogenesis. Sulfonylureas may also enhance synthesis of glucose transporters in adipocytes[35].

Metformin produced a significant reduction in blood glucose level in diabetic rats, which is in agreement with other reports[31,37]. The hypoglycemic effect of metformin is mainly due to extrapancreatic effects[36,8]. Bailey[38] suggested that, metformin lowers the rate of intestinal absorption of glucose uptake by muscles and adipose tissues and stimulates glycogenesis by muscles. Also, Metformin decreases glucose production by hepatocytes through inhibition of gluconeogenesis[39] and stimulation of liver glycolysis[40].

The present work showed an unexpected significant elevation in both fructosamine and HbA_1 levels after thirty days of treatment by both chlorpropamide and glipizide. These results has been explained by Rendell et al[41], who suggested that, in alloxan diabetic rabbits, the concentration of fructosamine was plateaued four weeks after alloxan

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Table (1): Effect of chlorpropamide, glipizide and metformin on serum fructosamine level in diabetic rats after 10 and 30 days.

(Mean ± S.E.) **n = 6**

Treatment	Fructosamine (mmol/l)		
	Before treatment (control)	After treatment	
		10 days	30 days
Chlorpropamide (5 mg/kg).	2.60 ± 0.13	2.45 ± 0.11 (94.23) %	* a 3.18 ± 0.22 (122.31) %
Glipizide (2.5 mg/kg).	2.06 ± 0.08	2.30 ± 0.21 (111.65) %	** 2.99 ± 0.26 (145.15) %
Metformin (350 mg/kg).	1.79 ± 0.06	1.64 ± 0.07 (91.11) %	1.70 ± 0.03 (94.44) %

* : Significantly different from control value at $P < 0.05$.
** : Significantly different from control value at $P < 0.01$.
a : Significantly different from the value 10 days after treatment at $P < 0.05$.

Table (2): Effect of chlorpropamide, glipizide and metformin on glycolated haemoglobin level in diabetic rats after 10 and 30 days.

(Mean ± S.E.) **n = 6**

Treatment	Glycolatedhame haemoglobin		
	Before treatment (control)	After treatment	
		10 days	30 days
Chlorpropamide (5 mg/kg).	7.9 ± 0.69	8.39 ± 0.40 (106.07) %	* a 10.36 ± 0.62 (130.97) %
Glipizide (2.5 mg/kg).	7.04 ± 0.13	* 7.73 ± 0.27 (109.80) %	** a 9.37 ± 0.56 (133.10) %
Metformin (350 mg/kg).	7.05 ± 0.36	6.35 ± 0.33 (90.07) %	a 7.69 ± 0.26 (109.08) %

* : Significantly different from control value at $P < 0.05$.
** : Significantly different from control value at $P < 0.01$.
a : Significantly different from the value 10 days after treatment at $P < 0.05$.

Table (3): Effect of chlorpropamide, glipizide and metformin on triiodothyronine (T_3) level in diabetic rats after 10 and 30 days.

(Mean ± S.E.) **n = 6**

Treatment	Triiodothyronine T_3 (ng/ml)		
	Before treatment (control)	After treatment	
		10 days	30 days
Chlorpropamide (5 mg/kg).	0.63 ± 0.04	0.60 ± 0.05 (95.24) %	** a 0.38 ± 0.05 (60.32) %
Glipizide (2.5 mg/kg).	0.65 ± 0.07	** 0.36 ± 0.04 (55.39) %	** 0.35 ± 0.05 (53.85) %
Metformin (350 mg/kg).	0.83 ± 0.04	** 0.64 ± 0.4 (77.11) %	** a 0.51 ± 0.01 (61.45) %

** : Significantly different from control valuc at P < 0.01.
a : Significantly different from the value 10 days after treatment at P < 0.05.

Table (4): Effect of chlorpropamide, glipizidc and metformin on thyroxine (T_4) level in diabetic rats after 10 and 30 days.

(Mean ± S.E.) **n = 6**

Treatment	Thyroxine t_4 (ug%)		
	Before treatment (control)	After treatment	
		10 days	30 days
Chlorpropamide (5 mg/kg).	13.87 ± 1.07	** 19.01 ± 1.42 (137.60) %	* 18.57 ± 1.47 (133.89) %
Glipizide (2.5 mg/kg).	14.83 ± 1.30	** 25.32 ± 1.49 (170.74) %	** 22.87 ± 1.44 (154.21) %
Metformin (350 mg/kg).	19.4 ± 1.43	** 24.27 ± 1.83 (124.72) %	** 22.33 ± 1.87 (114.75) %

* : Significantly different from control value at P < 0.05.
** : Significantly different from control value at P < 0.01.

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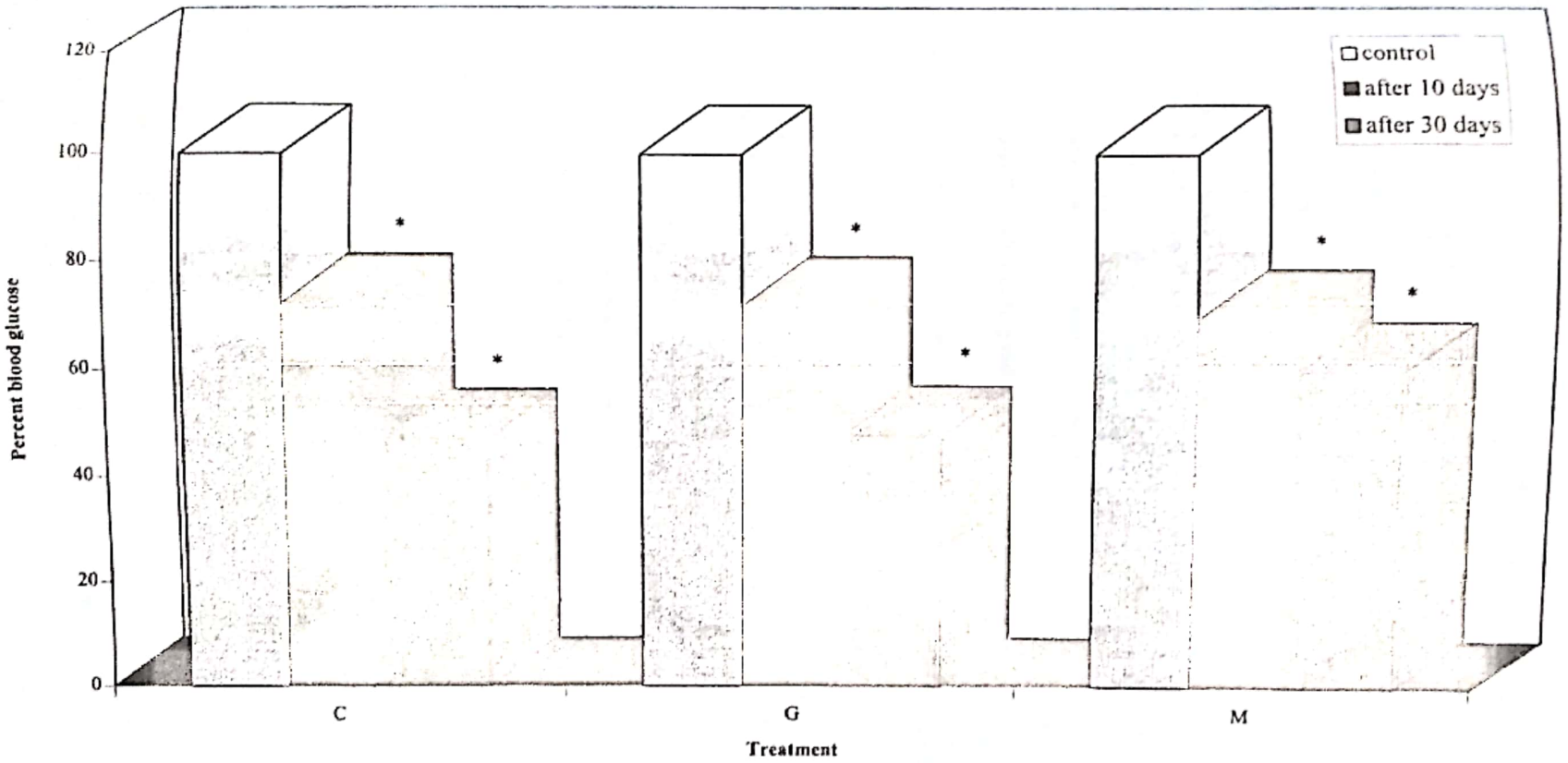

Figure: Percent effect of chorpropamide (C) (5 mg/kg), glipizide (G) (2.5 mg/kg) and metformin (M) (350 mg/kg) on blood glucose level in diabetic rats.

* : Significantly different from control value at $P < 0.05$

administration, while the concentration of HbA_1 was still rising. Also, In the present work, diabetes was induced in rats by S.C. injection of alloxan. Three days after alloxan injection, blood samples were collected to record parameters before treatment. The duration of diabetes induction was too short to reach the levels of fructosamine and HbA_1 in diabetic animals, where they were close to normal levels. By the progression of the experiment, the levels of fructosamine and HbA_1 were plateaued corresponding to blood glucose level in the treated groups.

Thus, Metformin has non-significant effect on both fructosamine and HbA_1 levels.

The present results revealed that, both chlorpropamide and glipizide significantly lowered T_3 level after 30 days and increased the level of T_4 significantly after 10 and 30 days in diabetic rats. This is in agreement with Hershman et al.[43], who reported that, sulfonylureas interfere with binding of T_3 and T_4 to thyroid binding globulins (TBG) by competitive inhibition. The reduction of T_3 by the effect of antidiabetic drugs may be due to decreased peripheral deiodination of T_4[43]. Also, the insulinotropic effect of sulfonylureas may elevate T_4 level according to Takiguchi et al.[44], who suggested that insulin treatment reversed the decrease of plasma thyroid hormone levels. Metformin lowered the T_3 level significantly after 10 and 30 days, but T_4 level showed non-significant increase. This result is in agreement with Kucharzeweski et al.[45], who suggested that in type II diatetics treated with oral hypoglycemics, T_3 concentration was reduced significantly while T_4 concentration did not differ significantly. The reduction in T_3 level may be attributed to inhibition of peripheral conversion of T_4 to T_3[43].

In conclusion, treatment of diabetic rats with chlorpropamide and glipizide decrease T_3 level and increase T_4 level, while treatment with metformin lower T_3 level and has limited effect on T_4 level, indicating a modulatory action of these antidiabetic drugs on thyroid function.

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التأثير المغير للأدوية المضادة لمرض السكر على وظائف الغدة الدرقية في الفئران المصابة بالبول السكرى

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أعد هذا البحث لدراسة تأثير علاج مرض البول السكرى (المستحدث فى الفئران) بإستخدام الكلوربروباميد (٥مجم/كجم/يوم) والجليبيزايد (٥ر٢ مجم/كجم/يوم) والمتفورمين (٣٥٠مجم/كجم/يوم) على وظائف الغدة الدرقية ممثلة فى هرمونى الثايروكسين وثلاثى يودو الثيرونين - كما تم دراسة تأثير هذه الأدوية على مستوى السكر والهيموجلوبين الجليكولى والمتهيموجلوبين فى مصل الدم. أحدث كل من الأدوية المذكورة انخفاضاً ملحوظاً فى نسبة السكر فى الدم بعد ١٠، ٣٠ يوم من إعطاء الأدوية للفئران. بينما إرتفعت نسبة كل من الهيموجلوبين اللجليكولى والفركتوزاين بعد ٣٠ يوم من اعطاء كل من الكلوربروباميد والجليبزايد. ولم يحدث المتفورمين أى تغيير فى هذين المؤشرين. انخفضت نسبة ثلاثى يودوالثيرونين بينما أرتفعت نسبة الثيروكسين بدرجة ملحوظة بعد ٣٠ يوم من إعطاء الثلاث عقاقير تحت الدراسة. مما سبق نستنتج أن كل من الكلوربروباميد والجليبزايد والمتفورين يخفض من مستوى السكر بالدم فى الفئران المصابة بمرض السكر. بينما لم يعبر التغير الذى حدث فى نسبة الفركتوزأمين والهيموجلوبين الجليكولى عن الإنخفاض الحادث فى نسبة السكر نتيجة للعلاج بهذه العقاقير. وهذا يمكن تفسيره بأن مدة الثلاثين يوماً فى هذه الدراسة لم تغطى الفترة المطلوبة لحدوث مثل هذا الإرتباط بين هذه المؤشرات. أحدث كل من الجليبزايد والكلوربروباميد تغييراً فى وظائف الغدة الدرقية ممثلاً فى خفض مستوى هرمونى ثلاثى يودوالثيرونين ورفع نسبة الثيروكسين فى مصل الدم بينما لم يؤثر اعطاء المتفورمين على نسبة هرمون الثيروكسين فى الفئران المصابة بمرض السكر.