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Research Article

Synthesis and Study the Effect of Pyrimidine Derivative on Renal Function in Female Rats with Diabetes

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Abstract

A pyrimidine derivative was synthesized by reaction of beta- diketone (methyl 3-chloro-3-oxopropanoate) and quinidine to produce 6-chloro-4-methoxy-1, 2 primidine-2-amine in good yield. The current experience was carried out to estimate the effect of pyrimidine derivative (6-chloro-4-methoxy-1, 2-pyrimidin-2-amine) in the mitigating of the complication induced hyperglycemia (DM), as nephropathy of the female rats. Thirty two of the healthy female rats were used and randomly distribution into 4 groups and the treatment continued for 28 days that given daily, group 1 this used as control contained healthy rats was given 0.5 ml Dimethyl Sulphoxide (DMSO) intraperitoneal, group 2 contained healthy rats was given (46.86 mg/kg) of pyrimidine dissolved by 0.5 ml (DMSO) intraperitoneal, group 3 considered as positive control contained alloxanized rats was given 0.5 ml Dimethyl Sulphoxide (DMSO) intraperitoneal, whereas group 4 contained diabetic rats was treated with (46.86 mg/kg) of pyrimidine dissolved by 0.5 ml (DMSO) intraperitoneal. The results of the present study revealed that injection of alloxan resulted in elevation creatinine, urea and uric acid levels, in addition decrease in total protein accompanied by decrease in albumin and globulin levels, whereas the treatment with pyrimidine derivative lead to decreased of creatinine, urea and uric acid level as well as ameliorating total protein concurrently with albumin and globulin levels were markedly. The study demonstrated that the pyrimidine derivative repaired the kidney function and ameliorating renal function indices to normal limits in diabetic female rats.

Keywords: Creatinine; Uric acid; Urea; Pyrimidine derivative; Diabetes

Introduction

Diabetes mellitus; is a metabolic syndrome causing from defect in secretion of insulin or loss of tissue sensitivity to insulin, DM which has a significant adverse effect on vital organs such as heart, kidneys, eyes, and peripheral nerves as well as reproductive dysfunction [1,2]. Type I produces from immune mediated pancreatic β -cell damage causing to insulin deficiency known as insulin-dependent diabetes mellitus, while type II is the most common produces form insulin resistance and disease known as non-insulin-dependent diabetes mellitus, it is characterized by chronic hyperglycemia and hyperinsulinemia [3,4]. Hyperglycemia in DM causes high level of Reactive Oxygen Species (ROS) formation known as free radicals and inflammatory mediators thereby creating oxidative stress that results in the development of pathological states, the diabetes is one of these conditions [5,6]. The oxidative stress is an imbalance among the levels of the pro-oxidants and antioxidants in the biological systems, the variation in their levels makes the tissues more susceptible to oxidative stress which leading to cellular injury by lipids peroxidation of membrane [7]. The current research was carried out to the estimate ameliorate effect of pyrimidine derivative on the renal function in female rats with diabetes.

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The Median Lethal Dose LD_{50}

The acute median lethal dose LD_{50} of the synthesized compound after conducting a sequence of test levels when the compound was given Intraperitoneally (IP) to rats aging from (10-14) weeks, 200 g to 220 g weights using the up-and down method were observed for the appearance of any sign of toxicities for 24-hours lethality [8]. A sequence of trails was carried out with an equal spacing between doses using this method; elevated dose if the injected animal was not died and decreased dose if the injected animal was died. LD_{50} were depending on the final result reading response-dead (X) or non-response-lived (O). Then applied the following equation LD_{50}=XF+Kd.

The calculate of LD_{50} is XF+Kd, where (XF) is the final test level, (K) is the tabulated value and (d) is the interval between each two doses (Table 1).

LD_{50}=XF+Kd

LD_{50}=Median Lethal Dose

XF=Last dose used in the experiment

K=Change factor from the table

Table 1: Dixon values. Dixon et al. [8].

	K represented serial tests started with:-				
	O	OO	OOO	OOOO	
XOOO	-0.157	-0.154	-0.154	-0.154	OXXX
XOOX	-0.878	-0.861	-0.860	-0.860	OXXO
XOXO	0.701	0.747	0.741	0.741	OXOX
XOXX	0.084	0.169	0.181	0.182	OXOO
XXOO	0.305	0.372	0.38	0.381	OOXX
XXOX	-0.305	0.169	-0.144	-0.142	OOXO
XXXO	1.288	1.5	1.544	-1.549	OOOX
XXXX	0.555	0.0897	0.985	1	OOOO
	X	XX	XXX	XXXX	
	K represented serial tests started with:-				

d=Difference between doses

O=Symbol of survival animal after 24 hours of dosing

X=Symbol of dead animal after 24 hours of dosing

Synthesis of 6-chloro-4-methoxy-1,2-pyrimidin-2-amine

1.36 g (10 mmol) of methyl 3-chloro-3-oxopropanoatewith hydrazine hydrate was produced in equal molar ratios with a small increase in moles of quinidine and in the presence of dimethylformamide to produce the pyrimidine derivative with a good yield through the use of thin layer chromatography technique to reach the best conditions to obtain good productivity, as in the Figure 1.

The physiological study

Experimental design: 32 female rats with 200 g to 220 g weight, randomly divided into 4 groups of 8 rats each, 16 rats after overnight fasting, rats were injected intraperitoneally a single dose of 125 mg/kg B.W of alloxan according to Al-Fartosi and Hussein [9], dissolved in 1 ml normal saline. After 3 days of alloxan treatment, blood glucose level was measured by an easy glucometer, the average of blood sugar was more than 200 mg/dl in fasted rats were represented diabetic rats, these groups as following:

Group 1 this used as control contained healthy rats was given 0.5 ml Dimethyl Sulphoxide (DMSO) intraperitoneal, group 2 contained healthy rats was given 1/10 LD_{50} (46.86 mg/kg) of 6-chloro-4-methoxy-1, 2-dihydropyrimidin-2-amine dissolved by 0.5 ml (DMSO) intraperitoneal [10], group 3 considered as positive control contained alloxanized rats was given 0.5 ml Dimethyl Sulphoxide (DMSO) intraperitoneal, whereas group 4 contained diabetic rats was treated with 1/10 LD_{50} of pyrimidine (46.86 mg/kg) was dissolved with 0.5 ml (DMSO) intraperitoneal, all group treated for 28 days respectively. At the end treatment period (28 days), the animals were fasted overnight and anesthetized with chloroform, the blood was collected in plain tubes and left to clot, and serum was separated and kept at -20°C till used.

Assessment of renal function: Serum creatinine estimated using the method [11], urea was evaluated using the method described by Patton and Crouch [12], uric acid was carried out using [13], Total proteins was measured according to Henry [14], albumin was assayed by method [15], globulin according to Serum globulin=Serum total protein-Serum albumin.

Analysis of statistical: Data are shown as means ± SE; data analyzed by using one away variance analysis ANOVA test. Was used to comparison a criterion in different studied groups, the value of significance was set at $p \leq 0.05$.

Results

Chemistry

The reaction of beta-diketone (methyl 3-chloro-3-oxopropanoate) with quinidine in the presence of hydrazine hydrates in dimethylformamide to produce the pyrimidine derivative with a good yield, (Figure 1).

The fingerprint and other parts of the IR spectra for the synthesized molecule showed common features in some places and characteristic bands in others. The presence of the amino group (NH_2) stretching with a sharp area about 3550 cm^{-1} -3323 cm^{-1}.

The IR spectrum of synthesized compound show strong bands to C-H in pyrimidine ring at 3102 cm^{-1} and for C=C and C=N at 1659, 1593 cm^{-1} respectively, (Figure 2).

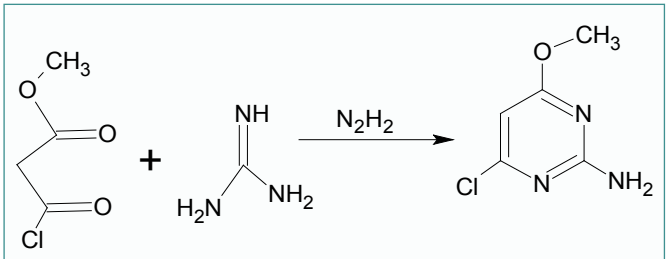

Figure 1: Preparation of 6-chloro-4-methoxy-1,2-pyrimidin-2-amine.

The signal attributable to methoxy protons (OCH_3) is seen at 3.81 ppm in the ^1H NMR spectra of the produced molecule., ^1H NMR spectra of Schiff base show a doublet at δ 6.05 ppm due to NH_2 proton and singlet at 7.04 ppm due to pyrimidine ring, (Figure 3).

The ^{13}C NMR spectra of synthesized compound were measured in DMSO-d_6. The spectra revealed the presence of C-OCH_3 group at δ 171.3 ppm. The signals at the range δ 163.3 and 160.2 ppm due to C-NH_2 and C=N respectively. The signal at δ 94.63 and 160.2 ppm due to pyrimidine ring. The signal at δ 54.09 ppm due to C-OCH_3, (Figure 4). These spectra data sports the structure of synthesized compound.

Median Lethal Dose (LD_{50})

In vivo determination of the 50% of the lethal dose (LD_{50}) of the studied compound was detected in the rats by using the "up-and-down" procedure described by Dixon et al. [8]. In this experiment, 29 rats their aged (10-14) weeks, 200 g to 220 g weights were used. Graded doses IP were injected to each animal; a series of concentrations 300 mg/kg B.W., 350 mg/kg B.W., 400 mg/kg B.W., 450 mg/kg B.W. dissolved in 0.5 ml Dimethyl Sulphoxide (DMSO), were given and chosen with equal spacing (concentrations) between doses.

Mortality was recorded after 24 hours that each one animal is treated with one dose and after 24 hours which was recorded as O if the animal lives and then the treated dose increased. While X is listed for the death of the animal and then decreased the dose according to for the result of the animal the code which formed as being (OOOX) and according to Dixon value to get and the LD_{50} was determined according to the formula employed by Dixon et al. [8].

$LD_{50} = Xf + Kd$

$LD_{50} = 450 + 0.372 \times 50$

LD_{50}=468.6 mg/kg B.W.

1/10 of LD_{50}=46.86 mg/kg

Discussion

Kidney has an essential role in maintain of the chemistry composition of fluids in the body at optimal degree *via* acidification of urine and removal of metabolite waste, the elevation of blood glucose has been resulting in prompting and expansion of diabetic complications, the nephron-pathy is one of these complications this may be noted in diabetic patients [16]. The results in Table 2 demonstrated that diabetes impaired kidney function mainly as represent by an augmentation of serum levels of creatinine and urea in diabetic rats compared with control and treated diabetic groups that were notable. The explanation for this increased due to kidney damage as evidenced by declined glomerular filtration rate for metabolic waste products from blood and excreted them in the

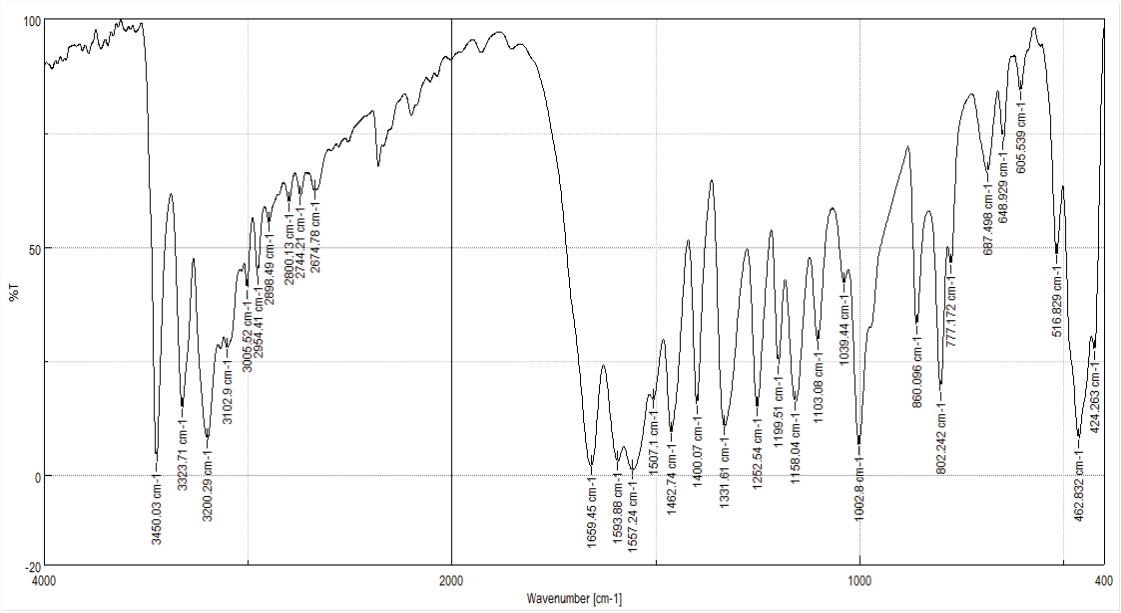

Figure 2: Infra-red spectrum of 6-chloro-4-methoxy-1, 2-pyrimidin-2-amine.

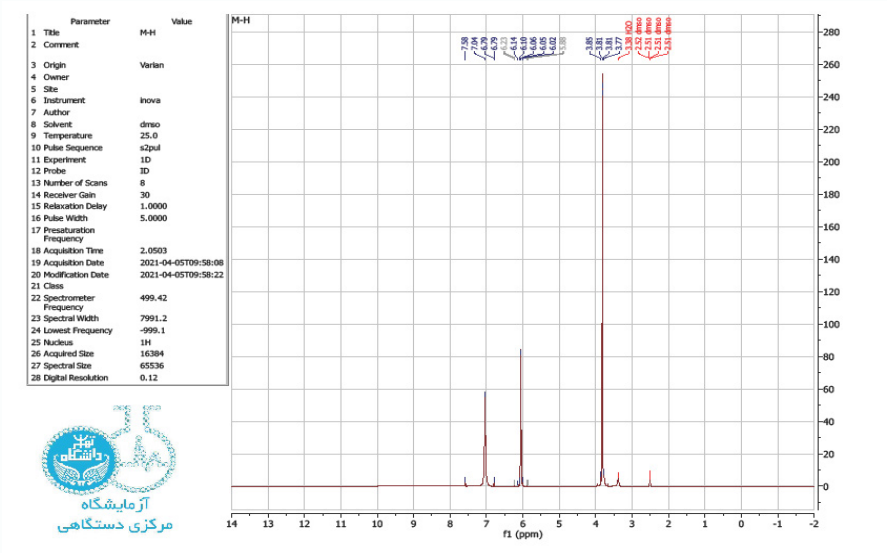

Figure 3: ^{1}H NMR spectrum of 6-chloro-4-methoxy-1, 2-pyrimidin-2-amine.

urine which are expressed a significant marker of renal dysfunction [17]. These findings are in agreement with the results obtained by Weam and Luma [18], showed that the mechanism of increased serum creatinine and urea levels in alloxan-induced diabetes could be attributed to the increased protein catabolism and renal dysfunction. The renal also has importance function in control of value of uric acid concentration in blood, so that it is simple excreted from the glomeruli to the tubule of kidney and it is reabsorbed which by the middle tubules (proximal convoluted), any change in the concentration of uric acid in fluids of the body indicate the defect of the organs function of the body Sharma et al. [19]. In present work the data indicate high level of uric acid in diabetic rats' comparison with control and treated diabetic rats, this may be attributed to 28 days of diabetes induction induces oxidative stress, glucose overload and glucose mediated osmotic diuresis these cause to impair the renal function [20]. This is in accordance with Lytvyn et al. [21], found that increased activity of renin-angiotensin-aldosterone system in diabetes causes a number of pathological alterations such as inflammation and high intraglomerular pressure resulting in renal damage. Also increased activity of renin-angiotensin-aldosterone system induced tubule interstitial fibrosis and afferent renal arteriolopathy inrodent models [22]. In same direction the data represented in diabetic effect in animals caused lowered in total protein concurrently with declined albumin and globulin. This alteration possible due to disturbance in protein anabolism and/or catabolism [23]. This is in accordance with Sharma et al. [24], and Hathama and Aymen [25], demonstrated similar result with the results in this study.

Results of the current experiment exhibited that continuous

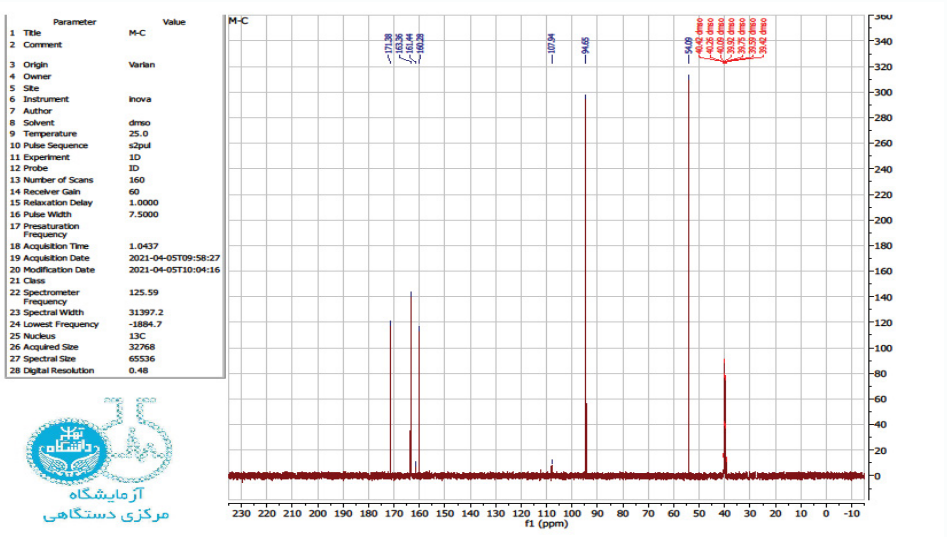

Figure 4: ^{13}C NMR spectrum of 6-chloro-4-methoxy-1, 2-pyrimidin-2-amine.

Table 2: Creatinine, urea, uric acid, total protein, albumin and globulin in normal control, non-diabetic, diabetic and treated diabetic rats with pyrimidine groups.

Groups	Creatinine mg/dl	Urea mg/dl	Uric acid mg/dl	Total protein g/dl	Albumin g/dl	Globulin g/dl
Control DMSO 0.5 ml	1.42 ± 0.01 B	33.32 ± 1.21 C	3.86 ± 0.21 B	5.87 ± 0.04 B	3.25 ± 0.01 C	2.89 ± 0.02 B
Non-diabetic rats treated 46.86 mg/kg of pyrimidine	0.98 ± 0.01 C	30.54 ± 1.14 D	2.90 ± 0.10 C	6.48 ± 0.05 A	3.65 ± 0.01 A	3.19 ± 0.01 A
Diabetic rats	2.44 ± 0.02 A	47.23 ± 2.13 A	4.76 ± 0.24 A	4.82 ± 0.03 C	2.97 ± 0.02 D	1.67 ± 0.02 D
Diabetic rats treated with 46.86 mg/kg of pyrimidine	1.52 ± 0.01 B	38.11 ± 1.44 B	40.12 ± 0.31 B	5.65 ± 0.02 B	3.43 ± 0.03 B	2.65 ± 0.01 C

Data are represented as ± SEM, the difference capital litters which are expressed significantly difference at (P<0.05) between control and experimental groups.

injection of pyrimidine derivative to diabetic animal daily for 28 days cause to enhanced the ability of the kidneys to remove waste products from the blood this is evidenced by lowering in serum creatinine, urea and uric acid levels, as well as improved total protein, albumin and globulin levels. The effect of pyrimidine derivative in this study may be attributed to effect of pyrimidine on thyroid hormones this explanation is in agreement with Fatima et al. [26], who observed the pyrimidine derivatives exerted on thyroid hormones and its synthesis. Also, in accordance with work by Bano et al. [27], that showed that pyrimidine derivatives have a free radical scavenging properties which provides organs protection against diabetes.

Conclusion

The study demonstrated that the pyrimidine derivative ameliorating the kidney function and restored elevation of renal function indices to normal limits in diabetic female rats.

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