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Characterization of Novel Formazan Derivative and Study its Activity of Antihyperglycemic

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Abstract

In the present research, a 1-[4-((E)-((Z)-(phenylimino) (3,4,5-trihydroxyphenyl) methyl)diazenyl) phenyl] ethanone, (a Formazan Derivative) was synthesized by the condensation of Schiff base (5-[(phenylimino)methyl]benzene-1,2,3-triol) and diazonium salt of 4-amino acetophenone. The Schiff base 5-[(phenylimino)methyl]benzene-1,2,3-triol was itself synthesized by the condensation of aniline with 3,4,5-trihydroxybenzaldehyde. All the reaction were monitored and purity was determined on TLC, and spots were visualized by exposing the dry plates in iodine vapours. The synthesized formazan derivative has been characterized by CHN, UV-Vis, IR spectral data and melting points.

The study was conducted to evaluate hypoglycemic effect of the synthesized formazan derivative on blood glucose level following oral administration to normal and alloxan treated rabbits. The rabbits are divided into control and treatment groups every group considered from six rabbits. The hypoglycemic effect of synthesized formazan derivative was studied in both types of rabbits. Group I was control group and received orally 3 ml of distilled water only. The groups II was treatment group and received orally with 150 mg/kg of synthesized formazan derivative dissolved in 3 ml of distilled water. Group III was treatment group too and received orally with 300 mg/kg of synthesized formazan derivative dissolved in 3 ml of distilled water.

The synthesized formazan derivative is recorded excellent hypoglycemic effect in the blood glucose level . The cellular toxicity of synthesized formazan derivative was studied and showed it is nontoxic.

Keywords . Formazan derivative, Antihyperglycemic effect, Diabetes mellitus, Cellular toxicity

Introduction

Formazans are compounds which contain the characteristic chain of atoms N=N–C=N–NH [1], Formazans have been found to possess important medical applications due to their various activities [2] such as antimicrobial [3,4], analgesic [5], antifungal [6], anticancer, anti-HIV [7], etc. Several formazans showed promising anti-fertility[8], anti-parkinsonian [9] and anticonvulsant activities [10]. Our idea was to combine Azomethine group (−CH=N−) and azo group (−N=N−) in one single molecule to get formazan derivative.

Diabetes mellitus, an endocrine disorder, is affects a large number of people worldwide. By the year 2010, the total number of people worldwide with diabetes is projected to reach 239 millions [11]. In modern medicine, no satisfactory effective therapy is still available to cure diabetes mellitus [12].

Diabetes mellitus is characterized by chronic hyperglycaemia and glucosuria produced by an absolute or relative insufficiency of insulin. The ailment may result into the development of further metabolic and anatomic disturbances among which is Lipemia, hypercholesterelaemia, loss of weight, ketosis, arteriosclerosis, gangrene, pathologic changes in the eye, neuropathy, renal disease and coma[13,14]. Management of diabetes mellitus sans side effects is still a challenge for the pharmaceutical world[15].

Materials and Methods

1- Materials and Instruments

All chemicals used in the present investigation were supplied from BDH and Fluka, Glucose estimation kit was supplied from Diachem Ltd (Hungaria).

The purity of the synthesized formazan derivative was checked by thin layer chromatography (TLC). The infrared (IR) spectra of synthesized formazan derivative was recorded on Shimadzu FT-IR spectrophotometer. The ultra violet-visible (UV-Vis) spectra of synthesized formazan derivative was recorded on Spectro Scan 80D spectrophotometer. The elemental analysis (CHN) data of synthesized formazan derivative was recorded on Euro vector EA 3000A Element analyzer. The melting points was determined in open Capillary tubes using Electrothermal (Gallen Kamp) apparatus.

2- Synthesis of formazan derivative

Synthesis of Schiff bases

A mixture of equimolar amount (0.1 mol) of aniline and 3,4,5-trihydroxyphenyl in ethanol (100 ml) and glacial acetic acid (10 drop) was refluxed for 3 hrs on water bath. The reaction mixture was concentrated ,cooled the solid obtained was filtered and recrystallized from ethanol to give schiff bases of 5-[(phenylimino)methyl]benzene-1,2,3-triol [16].

Diazotization of 4-amino acetophenone

The diazonium salt (4-acetylbenzenediazonium chloride) solution can be prepared as below:

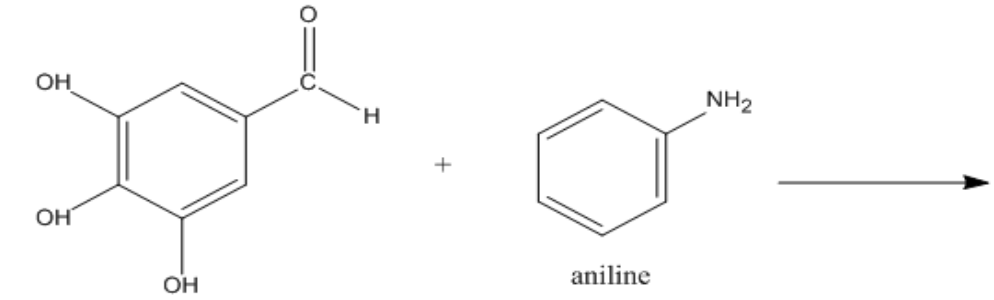
(a) Dissolve (0.01 mol) of $NaNO_2$ in 5 ml of water.

(b) Put (0.01 mol) of 4-amino acetophenone into 70 ml of water. Add slowly 12 ml of concentrated hydrochloric acid and stir the mixture.

(c) Cool the 4-amino acetophenone solution in an ice-bath. While keeping the solution at 0 °C add the sodium nitrate solution slowly with a dropper. The mixture should be stirred during addition. When the addition is completed, stir the mixture for another 2-3 minutes [17].

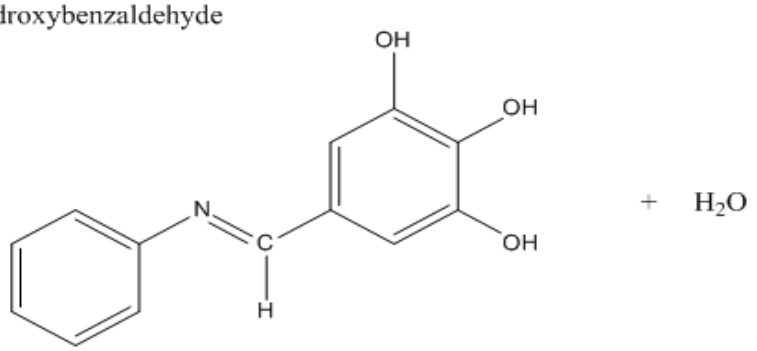
Synthesis of formazan derivative

The Solution of Schiff bases (0.1 mole) in pyridine (70 ml) was reacted with cold diazonium chloride of 4-amino acetophenone (0.1 mole) in the presence of sodium acetate (2 gm) in ice bath at 0-5 °C for 3 hour colored product obtained was filtered and washed with water till it was free from excess pyridine and crystallized from ethanol [18]. Formazan derivative were synthesized according to following scheme 1. The physical and analytical data obtained for these compounds are shown in table 1.

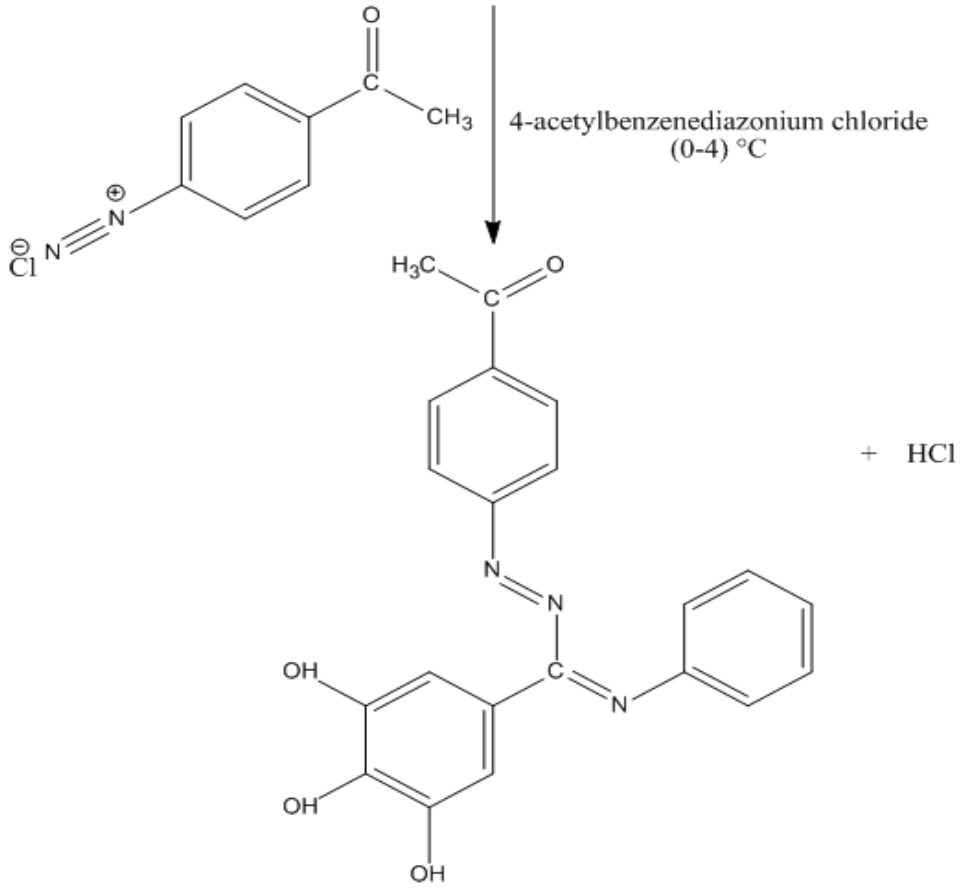


3,4,5-trihydroxybenzaldehyde

aniline



5-[(phenylimino)methyl]benzene-1,2,3-triol
Schiff Base



1-(4-((*E*)-((*Z*)-(phenylimino)(3,4,5-trihydroxyphenyl)methyl)diazenyl)phenyl)ethanone

Scheme 1: synthesized formazan derivative

Table 1: The physical and analytical data for synthesized compound

Compound	Molecular formula	Molecular weight	Color	Physical state	M.P. °C	λ_{max} (nm)	R_f
Synthesized compounds	$C_{21}H_{17}N_3O_4$	375.38	Yellow	Powder	242-244 °C	416	0.83

3- Animals

Healthy male rabbits of local strain, weighing 1300-1500g were used in these experiments. Before using the rabbit for experiment, rabbits were kept under observation for a week in animal house. The animals were offered a balanced rabbits diet consisting of green leaves, fodder and water.

4- Preparation of Diabetic rabbits

Rabbits were made diabetic by injecting 150 mg/kg body weight of alloxan monohydrate intravenously for three days, the alloxan monohydrate was dissolved in 0.5 ml distilled water [19]. Eight days after injecting the alloxan monohydrate, blood glucose levels of surviving rabbits were determined by glucose oxidase method. Rabbits with blood glucose levels between 340-390 mg/100 ml were considered diabetic and employed in the study. Alloxan monohydrate has been observed to cause a massive reduction of the β-cells of the islets of Langerhans and induce hyperglycemic [20].

5- Determination of cellular toxicity using sheep erythrocytes

The method described by Xian-guo and Ursula [21] was employed to study cellular toxicity. Briefly, 10-fold serial dilutions of the (400 mg/ml) synthesized formazan derivative were made in phosphate buffered saline (400, 350, 300, 250, 200, 150,, 100, 50, 25, 10) mg/ml. A total volume of 0.8 ml for each dilution was placed in an eppendorf tube. A negative control tube (containing saline only) and a positive control tube (containing synthesized formazan derivative) were also included in the analysis. Fresh sheep erythrocytes (Fresh blood) were added to each tube, to give a final volume of 1 ml. Solutions were incubated at 37C for 30 min and all tubes were centrifuged for 5 min and then observed for hemolysis. Complete hemolysis was seen by a clear red solution without any deposit of erythrocytes. Hemolysis was also checked microscopically for presence or absence of intact RBCs.

6-Collection of blood and biochemical determination

Blood was collected from the marginal vein of the rabbits using syringe from the marginal vein of the ear, 2, 4, 6, 8 and 24 hours after synthesized formazan derivative administration. Plasma was separated by centrifugation at 3000 rpm for 10 minutes. Plasma glucose levels were then measured by commercial kit by using a glucose oxidize method.

7- Synthesized formazan derivative administration

The amounts of synthesized formazan derivative was calculated for each rabbit on body weight basis 150 mg/kg and 300 mg/kg respectively [22] and prepared by dissolved in 3 ml of distilled water. The water and synthesized compound were administered to each rabbit orally using a syringe.

8- Determination of blood glucose

Blood glucose was determined by using Glucose GOD-PAP kit (Diachem Ltd Hungaria). The results obtained were very accurate by this method and gave true glucose determination.

9- Statistical Analysis

The data were subjected to analysis of variance [23] using SPSS 10.0 for Windows program. The data were analysed with ANOVA in SPSS.

Results and Discussion

1- Identification of synthesized formazan derivative

The thin layer chromatography (TLC) results showed only a single spot was observed for synthesized formazan derivative. The synthesized formazan derivative under study was identified by many techniques: IR, CHN and UV-Vis.

The IR spectra provide valuable information regarding the nature of functional group. The IR spectra of synthesized compound is shown in Scheme 2 and Table 2 :

According to Silverstein et al. & Pavia et al. [24,25] the stretching vibrations of active groups for the synthesized formazan derivative are appear as below:

1- Phenolic O–H in (3200-3600) cm^{-1}

2- Aromatic C–H higher than 3000 cm^{-1}

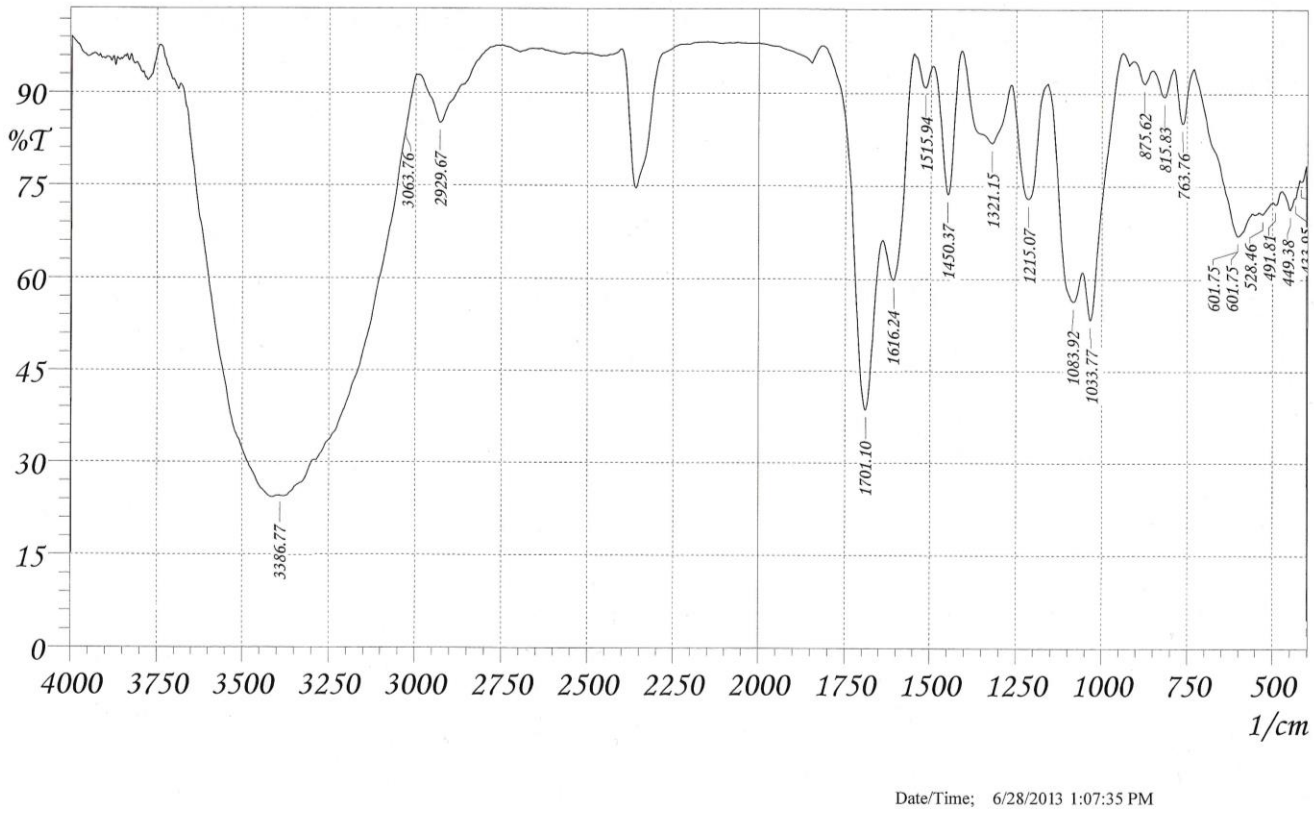
3- Aliphatic C–H less than 3000 cm^{-1}

4- C=O in (1640-1850) cm^{-1}

5- C=N in (1560-1640) cm^{-1}

6- N=N in (1480-1560) cm^{-1}

7- C=C in 1420-1480) cm^{-1}



Scheme 2: IR Spectra of synthesized formazan derivative

Table 2. IR spectral data of synthesized formazan derivative

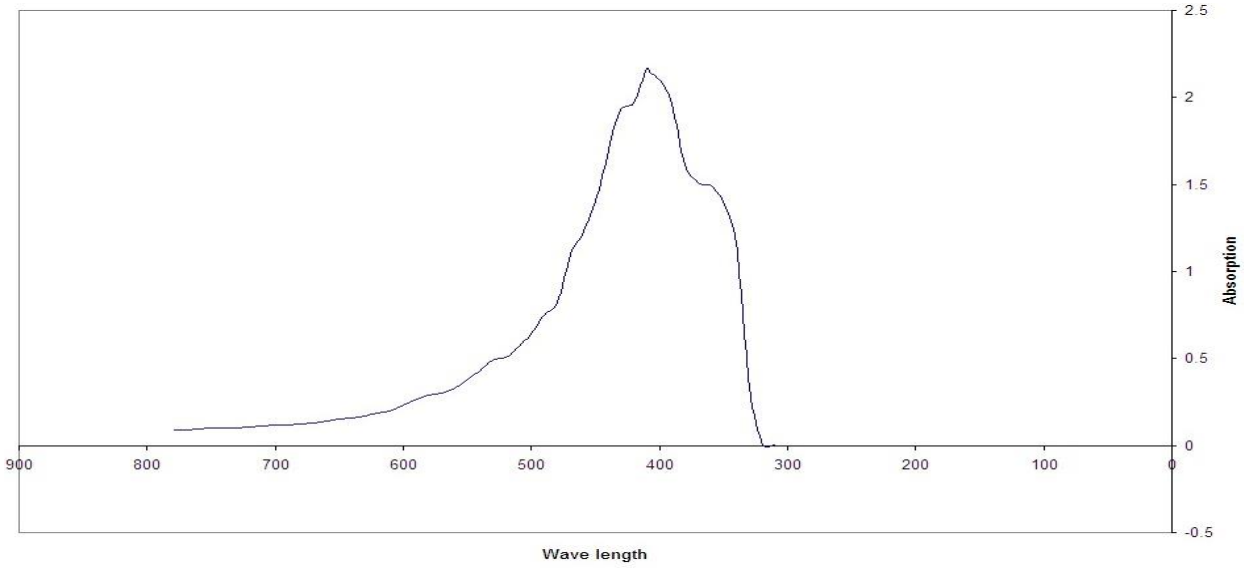
Compound	IR Bands	
	Frequency (cm^{-1})	Characteristics
Synthesized formazan derivative	3386.77	Phenolic O–H
	3063.76	Aromatic C–H
	2929.67	Aliphatic C–H
	1701.10	C=O
	1616.24	C=N
	1515.94	N=N
	1450.37	C=C
	1215.07	C–N
	1033.77	C–O

The results of element analysis CHN of synthesized formazan derivative is shown in Table 3:

Table 3. element analysis CHN of synthesized formazan derivative

Compound	C%		H%		N%	
	Calculated	Experimental	Calculated	Experimental	Calculated	Experimental
Synthesized formazan derivative	67.19	67.66	4.56	4.21	11.19	11.28

The results of ultra violet-visible spectra (UV-Vis.) of synthesized formazan derivative is shown in Scheme 3 :



Scheme 3 : (UV-Vis.) spectra of synthesized formazan derivative

2- Effect of synthesized formazan derivative on blood glucose levels in normal and diabetic rabbits

According to Quesenberry and Carpenter [26] and Kahn [27], rabbit's fasting glucose ranges from 75 mg/dl to 155 mg/dl, values very similar to those found in this study. Several studies have been carried out using experimental alloxan-induced diabetic rabbits. In these animals, fasting glucose was determined, being the values obtained very similar to ours. Thus, Annamala and Augusti [28] showed average values of 329.1 mg/dl and Lenich et al. [29] determined glucose values between 368 and 380 mg/dl. Schiller and McNamara [30] considered as hyperglycemic rabbits those with basal glucose between 170 and 400 mg/dl and diabetics when values were above 400 mg/dl.

The mean blood glucose concentration ± SEM of control and drug treated animals after oral administration of different doses of synthesized formazan derivative at various time intervals are summarized in Table 4,5,6 and Table 6.

Table 4 : Effect of synthesized formazan derivative (150 mg) on normal rabbits

Extract Dose (mg / kg)	N	Blood Glucose Level (mg/100 ml)				
		0 hrs	2 hrs	4 hrs	6 hrs	24 hrs
Control 3 ml distilled water	6	134.8 ± 4.5	131.5 ± 0.8	126.6 ± 4.6	121.3 ± 6.8	110.1 ± 2.6
150 Synthesized compound	6	133.3 ± 1.2	116.5** ± 3.8	99.8** ± 2.4	88.6*** ± 4	83.1*** ± 5.5

Blood Glucose Concentration = Mean±SEM

N = Number of Rabbits , **P<0.01 , ***P<0.001

The drug produced a significant decrease ($P < 0.05$) in blood glucose at 2 and 4 hours' intervals and the highly significant decrease ($P < 0.001$) was found at 12 and 24 hours interval.

Table 5: Effect of synthesized formazan derivative (300 mg) on normal rabbits

Extract Dose (mg / kg)	N	Blood Glucose Level (mg/100 ml)				
		0 hrs	2 hrs	4 hrs	6 hrs	24 hrs
Control 3 ml distilled water	6	134.6 ± 2.4	132.8 ± 3.9	128.8 ± 5	121.1 ± 3.5	109.1 ± 1.1
300 Synthesized compound	6	135.5 ± 2.2	110 *** ± 2	93.5*** ± 1.9	81.5*** ± 4.4	72*** ± 3.3

The drug produced a highly significant decrease ($P < 0.001$) was found at 2,6,12 and 24 hours interval

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Table 6 : Effect of synthesized formazan derivative (150 mg) on diabetic rabbits

Extract Dose (mg / kg)	N	Blood Glucose Level (mg/100 ml)				
		0 hrs	2 hrs	4 hrs	6 hrs	24 hrs
Control 3 ml distilled water	6	374.8 ± 4.3	359.3 ± 2	344.6 ± 4.3	330.5 ± 3.5	318.1 ± 2.8
150 Synthesized compound	6	373.8 ± 4.1	341.8** ± 3.3	300.1*** ± 2.1	245.5*** ± 3.1	190.3*** ± 6

The drug produced a significant decrease ($P < 0.01$) in blood glucose at 2 hours' intervals and the highly significant decrease ($P < 0.001$) was found at 6,12 and 24 hours interval.

Table 7 : Effect of synthesized formazan derivative (300 mg) on diabetic rabbits

Extract Dose (mg / kg)	N	Blood Glucose Level (mg/100 ml)				
		0 hrs	2 hrs	4 hrs	6 hrs	24 hrs
Control 3 ml distilled water	6	373.3 ± 1.6	363.6 ± 3	341.6 ± 2.8	331.5 ± 5.6	316.6 ± 4.8
300 Synthesized compound	6	372.8 ± 3	321*** ± 6.1	269.1*** ± 4.9	202.6*** ± 2.1	133.5*** ± 2.4

Alloxan has been observed to cause necrosis of β-cells and induce hyperglycemia. Intraperitoneal injection of alloxan after administration of high fat diet causes type 2 diabetes [31]. In type 2 diabetic rabbits treated with synthesized formazan derivative, a highly significant decrease ($P < 0.001$) was found at 2,6,12 and 24 hours.

Conclusion

Synthesis of formazan derivatives by the condensation of Schiff base and diazonium salt is the eased method to synthesis of formazan derivatives.

The effect of synthesized formazan derivative on normal and diabetic rabbits proved that the synthesized formazan derivative exhibited better antihyperglycemic property. synthesized formazan derivative show a highly significant decrease in blood glucose levels.

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تشخيص مشتق فورمازان ودراسة فعاليته المخفضة لسكر الدم

تاريخ الاستلام : 9\10\2014 **تاريخ القبول : 9\1\2014**

حسنين جمهور جاسم
جامعة المثنى ، كلية التربية الأساسية ، قسم العلوم العامة

الخلاصة

في هذا البحث حضر المركب 1-[4-((E)-((Z)-(phenylimino) (3,4,5-trihydroxyphenyl) methyl)diazenyl)phenyl]ethanone (مشتق للفورمازان) من خلال تكثيف قاعدة شف 5-[(phenylimino)methyl]benzene-1,2,3-triol] مع ملح الديازونيوم لـ 4-أمينو اسيتوفينون. حضرت قاعدة شف من خلال تكثيف الأنلين مع 3,4,5-ثلاثي هيدروكسي بنزلديهايد. تم متابعة جميع التفاعلات والنقاوة باستخدام TLC ، وتم إظهار البقع في TLC من خلال تعريض الصفائح الجافة لبخار اليود . شخص مشتق الفورمازان المحضر باستخدام CHN وطيف UV-Vis وطيف IR ودرجة الانصهار .

وقد أجريت الدراسة لتقييم التأثير المخفض لمشتق الفورمازان المحضر لمستوى سكر الدم بد التجريع الفموي للأرانب الطبيعية والأرانب المعالجة بالالوكسان، قسمت الأرانب إلى مجموعة سيطرة ومجموعة معالجة وكل مجموعة تتكون من ست أرانب. درس التأثير المخفض للسكر لمشتق الفورمازان المحضر. المجموعة الأولى كانت مجموعة سيطرة وأعطيت فمويا 3 مل فقط من الماء المقطر، المجموعة الثانية كانت مجموعة معاملة وأعطيت فمويا 150 ملغم/كم من مشتق الفورمازان المحضر مذابا في 3 مل من الماء المقطر، المجموعة الثالثة كانت أيضا مجموعة معاملة وأعطيت فمويا 300 ملغم/كم من مشتق الفورمازان المحضر مذابا في 3 مل من الماء المقطر.

سجل مشتق الفورمازان تأثيرا مخفضا ممتازا لمستوى سكر الكلوكوز. درست السمية الخلوية لمشتق الفورمازان المحضر وثبت انه غير سام .

كلمات مفتاحيه: مشتق الفورمازان ، التأثير المخفض لسكر الدم ، مرض السكر ، السمية الخلوية .