

**EXTRACTION, ISOLATION AND IDENTIFICATION OF
SOME ALKALOIDS COMPOUNDS FROM IRAQI MEDICINAL
PLANT *HALOXYLON SALICORNICUM* AND STUDY
ANTIHYPERGLYCEMIC EFFECT IN ALLOXAN –INDUCED
DIABETIC RABBITS**

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ABSTRACT

The present study was carried out to determine and investigate the antihyperglycemic action of the alcoholic extract and alkaloids extracted from Iraqi Medicinal Plant *Haloxylon salicornicum* on blood glucose level of Alloxan –Induced Diabetic Rabbits (150 mg/kg intraperitoneal). In present study were prepared are alcoholic extract and isolated alkaloids. Preliminary qualitative tests were carried out for all prepared extracts and identification bio active chemicals alkaloid compounds (Acetic acid (amino carbonyl); 4-fluoro histamine; hydroxyl urea; n-hexyl methyl amine; 1-methyl dodecyl amine; Octadomine) by TLC and GC-MS chromatography. Alloxan –induced diabetic rabbits was prepared after injecting the experimental rabbit with three dose of alloxan (I.V). Alloxan –induced diabetic rabbit, the results indicated that alcoholic extract and alkaloids extracted from *Haloxylon salicornicum* showed significant decreasing in glucose conc. levels. Alkaloids isolated from *Haloxylon salicornicum* showed very high activity to decrease blood glucose levels in hyperglycemic rabbits, where significant decreasing ($P < 0.05$) was found at second hrs, significant decreasing ($P < 0.01$) at fourth hr. and high significant decreasing ($P < 0.001$) at sex and twenty fourth hrs. Both alcoholic extract

and alkaloids extracted *Haloxylon salicornicum* have potential hypoglycemic effect in hyperglycemic induced rabbits

INTRODUCTION

Diabetes Mellitus (DM) is the number one killer among all chronic diseases in the world (1) , by an increased concentration of blood glucose due to derangement in carbohydrates metabolism and defective secretion of insulin. The main symptom of DM is hyperglycemia, which leads to many complications classified into “micro vascular” and “macro vascular” , micro vascular complications, such as diabetic nephropathy and diabetic retinopathy, result from damages to the small blood vessels, whereas the macro vascular complications are caused by damages to arteries, leading to coronary artery and periphery artery diseases, and stroke. Basically, hyperglycemia is the result of relative insulin deficiency, insulin resistance or both (2, 3). diabetes mellitus (DM) is increasing across the world , reported showed that in the year 2000, there were about 171 million diabetes mellitus cases worldwide in patients ages 20 years or more, and in the year 2011 the World Health Organization (4) estimated that over 346 million of people live with DM worldwide. Nearly 80% of deaths due to DM occur in low and middle income countries Traditional herbal material plays an important part in the treatment of diabetes. Many herbal medicines have been recommended for the treatment of diabetes mellitus, medicinal plants have the advantage of having no side effects , in addition high cost of conventional treatments with synthetic drugs, traditional treatment with plants becomes an alternative option for financially poor populations , More than thirteen thousand plants have been studied for various pharmacological properties(5) .Therefore, the goal of pharmacotherapy is to normalize the blood glucose levels (6). *Haloxylon salicornicum* is a desert plant belongs to the family Chenopodiaceae, which has 120 genera and more than 1300 species. *H. salicornicum* is a branched shrub grows up to 1 m height. The new branches are green , succulent while the older ones are yellowish white to silvery white (7) , plant is known locally as Hamit and it is widely distributed in Iraq specialty southern of Iraq and It is distributed from Western Mediterranean region to Arabia, Iran, Mangolia , Burma and Southwest of China (8).

The plant is reported to be used as anti-diabetic (9), antibacterial (10) and anti-inflammatory (11). Two species of the genus were recorded in the literature to have folkloric uses. *H. salicornicum* is reported to be used for antiseptic and anti-inflammatory (9, 7). Traditional healers are using it to treat intestinal ulcers (12). In Oman the stems of this species are used as a mordant for dyeing wool in traditional weaving. In addition, *Haloxylon scoparium* {*Haloxylon articulatum*} is used to treat eye disorders (13). Infusion and powder infusion of aerial part of *H. scoparium* are used in Morocco for their antidiabetic effects (14,15). The qualitative phytochemical analysis of the aerial parts of the plant revealed, the presence of alkaloids, cardiac glycosides, anthraquinones, flavonoids, saponins, coumarins, sterols, tannins, volatile oils and volatile bases (9). On the other hand, few species of the genus *Haloxylon* (seven species) have been chemically investigated, which resulted in the isolation of the several alkaloids belonging to mainly seven classes of alkaloids. These classes are: aliphatic quaternary alkaloids, pyridine alkaloids, indole alkaloids, isoquinoline alkaloids, isoquinolone alkaloids, β -carboline alkaloids and phenyl ethylamine alkaloids (16). A piperidyl alkaloid "haloxynin" has also been isolated and characterized from *Haloxylon salicornicum* by mass spectrometry, among the 80 identified alkaloids. New flavonoids quercetin 3-*O*- β -glucosyl [1 \rightarrow 2]- α -rhaminoside-7-*O*- α -rhaminoside and quercetin 3-*O*-*p*-coumaryl [1 \rightarrow 6]- β -glucosyl [1 \rightarrow 6]- β -glucoside-7-*O*- α -rhaminoside, together with known compounds quercetin 3-gentiobioside, isoquercetin, quercitrin and kaempferol were isolated from the aerial part of *O. baccatus* (17).

MATERIAL AND METHODS

Plant collection and sampling

Plant Material. *H. salicornicum* were collected from South Nassira city, Southern of Iraq and identified at the department of Biology, college of Sciences, University of Thi-Qar, Iraq. The dried aerial parts plant in 25 C were powdered using a milling machine. The powder, weighing about 890 gm.

Preparation of Alcoholic Extracts

50 gm. of dried grounded aerial part were refluxed in 250 ml of 70% ethanol for 24 hours, the precipitate was removed by filtration, through filter paper no.1, then filtrate was concentrated under vacuum using freeze drier to afford (6.76) gm.

Isolation of Alkaloids and nitrogen compounds

50 gm. of defatted powder were mixed with 250 ml of 10% ethanol and acetic acid on magnetic stirrer for 24 h. Then filtered and concentrated to quarter of previous volume by using vacuum rotator evaporator at 70°C and the pH was adjusted to 9 with ammonium hydroxide to precipitate the alkaloids. The mixture was put in separation funnel, then 20 ml of chloroform was added and the mixture was mixed well. The organic layer was collected. This step was repeated three times then they were dried by vacuum using rotator evaporator to yield (2.30) gm. (18).

Column chromatography(CC)

column chromatography(CC). separation of Alkaloids and nitrogen compounds were carried out size-exclusion column chromatography over Sephadex using (CH₂Cl₂: MeOH, 1:1) using 10:90(Methanol :Di chloro methane) as the mobile phase(19).

Thin layer chromatography (TLC)

Thin layer chromatography (TLC) were used for isolation, separation and purification the active chemical compounds in biochemistry laboratories at chemistry department in college of sciences at university of Thi Qar in Iraq. TLC analysis was carried out on 0.2 mm silica gel, aluminium-backed plates (Merck Art.5554), by using solvent system Dichloromethane:Methanol (50:50). The plates were developed using (anisaldehyde: conc. H₂SO₄: methanol [1:2:97] spray reagent and heating(19).

Gas Chromatography Mass Spectrometry (GC-MS)

GC-Mass spectra were achieved by using a Hewlett Packard G1800A GCD system in Chemistry department, college of pure sciences education at University of Basra, Iraq. The alkaloid extract was dissolved in methanol depending on solubility, a 2 µl sample was injected then volatilized at 250°C. The column, a 30 m x 0.250 mm, 0.25 Å, HP-5-MS column, was heated initially to 50°C for 3 min and then ramped up to 250°C at 10°C per

minute for 2 minutes, the temperature was then held at 250°C for the remaining 20 minutes.

Phytochemical Analysis

Phytochemical qualitative analysis was performed using the general method for phytochemical screening of tannins, glycosides, flavonoids, alkaloids and saponins with modification (20).

Identification of Tannins

Half a gram of extract was stirred with 10 mL of distilled water and filtered. Four drops of a 1% ferric chloride solution were added to 2 mL of the filtrate. Blue-black, green or blue-green precipitate indicated the presence of tannins.

Identification of Glycosides

Half gram of extract was dissolved in 1 mL of distilled water. Aqueous NaOH 2M was added to the solution and the resulting yellow coloration indicated the presence of glycosides.

Identification of Flavonoids (Ferric Chloride Test)

Half gram of extract was boiled with 3 mL of distilled water. The solution was then filtered; and 2 mL of the filtrate were mixed with 5 drops of a 10% ferric chloride solution. The color change to green-blue or violet indicated the presence of the phenolic hydroxyl group.

Identification of Alkaloids (Dragendorff's Test)

Approximately 0.2 g of extract were stirred with 5 mL of 1% hydrochloric acid (HCl) in a water bath. The solution was filtered; and 1 mL of the filtrate was added to a test tube. Next, Dragendorff's Reagent was added to the filtrate. The presence of an orange color signaled the presence of alkaloids.

Identification of Saponins

Approximately 0.2 g of extract were dissolved in 5 mL of distilled water and the mixture was heated until boiling. The solution was then cooled and filtered. Next, 3mL of distilled water was added to the filtrate and the tube was shaken vigorously. Formation of froth indicated the presence of saponins.

Animals

Rabbits weighting 1.25-1.800 Kg were procured from local market in Basra , Iraq .Animals were kept in clean polypropylene cages and fed on standard antibiotic free diet. All animals were kept in fast for 24 hr. before starting the experiments .

Diabetes Induction of Rabbits

The rabbits were induced practically using three injections of alloxane monohydrate dissolved in sterile normal saline. After this process, rabbits became in diabetes case. Solution of alloxane was used immediately after preparation and was administrated at period of 48 hrs in a dose equal to 150mg/Kg body weight of rabbits and injected via marginal ear vein under light in 1ml syringe (21), then 20% of glucose dissolved in drinking water was given to rabbits (20% glucose) orally and they were kept in fast for 18 hrs after seven days from last administration. Glucose concentrations were measured in blood of alloxan-induced diabetic rabbits by using glucose oxidase peroxidase enzymatic colorimetric GOD-PAP method (22).

Effect of alcoholic extract and alkaloids on glucose level in alloxan –induced diabetic rabbits

Twelve fasted hyperglycemic rabbits were divided into two equal groups. The first was given 3ml of normal saline and while the second group was given 0.3gm/kg body weight of alcoholic extract dissolved in normal saline concentration was fitted as 100 mg /ml ,each rabbit dose 3ml /Kg BW which was considered as treatment group. Blood samples were collected at times (0 (as fasting), 2,4,6 and 24 hrs.). The glucose concentrations were measured at each time point (21,22).and same methods administration of 3 ml of 0.3gm/kg BW alkaloids extract.

RESULTS AND DISCUSSION

Yield of *Haloxylon salicornicum* Extracts

Percentages of *H. salicornicum* extracts were calculated , as illustrated in table (1)

Table(1). Percentages of *prosopis juliflora pods* extracts

No	Extract type	Yield (%)
1.	Alcoholic Extract	13.52
2.	Alkaloids	4.60

Qualitative Analysis of Alcoholic Extract and Alkaloids of *Haloxylon salicornicum*

The phytochemical analysis of *Haloxylon salicornicum* extracts plant showed the presence of major metabolites of saponins, alkaloids, tannins, glycosides and cardiac glycosides. Table (2) indicates qualitative analysis results of alcoholic and alkaloids extract of *Haloxylon salicornicum*. The number of positive signs indicates the intensity of the reactions that reflects the quantity of secondary metabolism active compounds present in the extract.

Table (2). Qualitative analysis of extracts of *Haloxylon salicornicum*

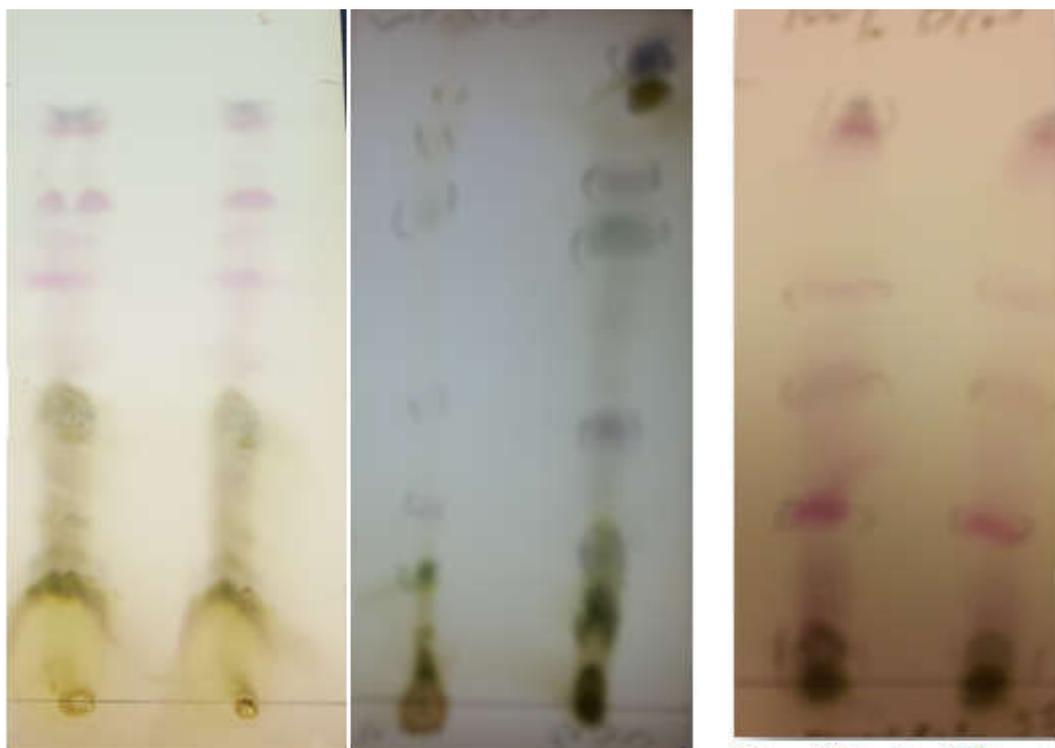
Reagents	Extracts		Results
	Alkaloids	Alcoholic	
Dragendroff	+++	+	alkaloids are present
Wagner	+++	+	
Mayer	+++	+	
Marquis	+++	+	
Molish	+++	+	Carbohydrates are present
Benedict	-	+	Glycosides are present in alcoholic extract
5% Hg Cl ₂	-	+	Saponin is present in alcoholic extract
Silver mirror	-	+	
Folin	-	+	Phenols are present in alcoholic extract
Alcoholic KOH	-	-	Flavonoids are absent in alcoholic extract
Mg- turnings	-	-	
1% Ninhydrin	-	++	Free amine groups and peptides are present in alcoholic extract
1% lead acetate	-	+	Tannins are present in alcoholic extract
1 % FeCl ₃	-	+	

+ positive test - negative test +++ quantitative presence

The preliminary phytochemical analysis of alcoholic extract of study plant (Table 2) showed the presence of a high number of phytochemicals. The presence of major metabolites of saponins, alkaloids, tannins, glycosides and cardiac glycosides. The number of positive signs indicates the intensity of the reactions that reflects the quantity of phytochemical present in the extract. (23,24,25,26)

Determination of alkaloids compounds from *Haloxylon salicornicum* by Thin Layer Chromatography (TLC)

Alkaloids and alcoholic extracts from *Haloxylon salicornicum* were separated on TLC and after spraying dragondorff reagent it was observed contains highest number of alkaloid with 6 spots giving positive alkaloid test. Active spots fractions separated by TLC showing in pictures(1 and 2) .



Picture (1) showing TLC spots of alcoholic extract from *Haloxylon salicornicum* plant

Picture (2) showing TLC spots of alkaloids from *Haloxylon salicornicum* plant

Pictures (1 and 2) indicate TLC results of alcoholic extract and alkaloids from *Haloxylon salicornicum* plant. Many chemical compounds of extracts because found many spots in TLC plates were have different R_f , but alkaloids has six nitrogen components, and this corresponded with extraction percentage of both extracts.

Gas liquid chromatography-mass spectrometry(GC-MS) of *Haloxylon salicornicum* Extract

Gas Chromatogram analysis for alkaloids identification six compounds were identified in the alkaloids extracted of *Haloxylon salicornicum*, this results of mass spectrum showing in table (3) and figures (1)-(6).

Table(3) the chemical constituents of *Haloxylon salicornicum* by GC–MS analysis

No.	Compound name	Molecular formula	m/z [M+H]
1	Acetic acid (amino carbonyl)	C3H4O4N2	+ 132
2	4-fluoro histamine	C5H8N3F	+ 129
3	hydroxyl urea	C H4N2O2	+ 76
4	n-hexyl methyl amine	C7H17N	+ 115
5	1-methyl dodecyl amine	C13H29N	+ 196
6	Octadomine	C8H19 N	+ 128

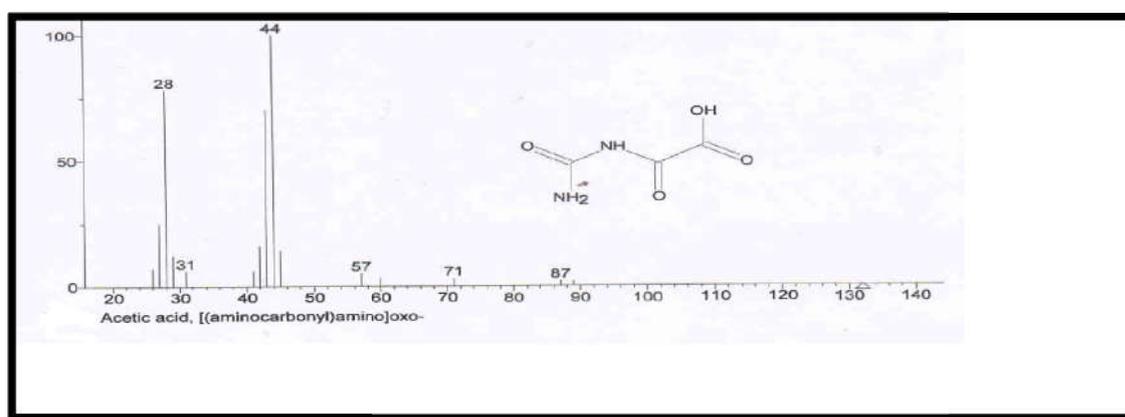


Fig.1 Mass Spectrum for Acetic acid (amino carbonyl)

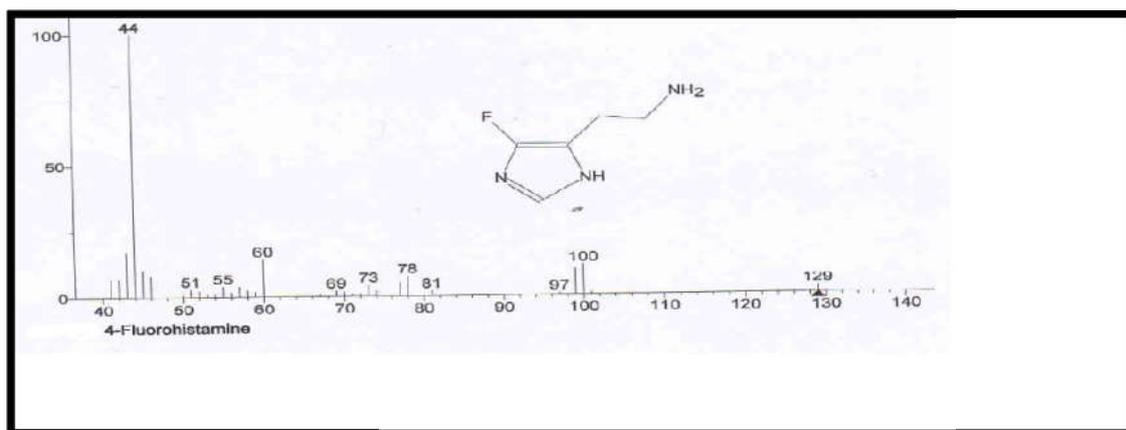


Fig.2 Mass Spectrum for 4-fluoro histamine

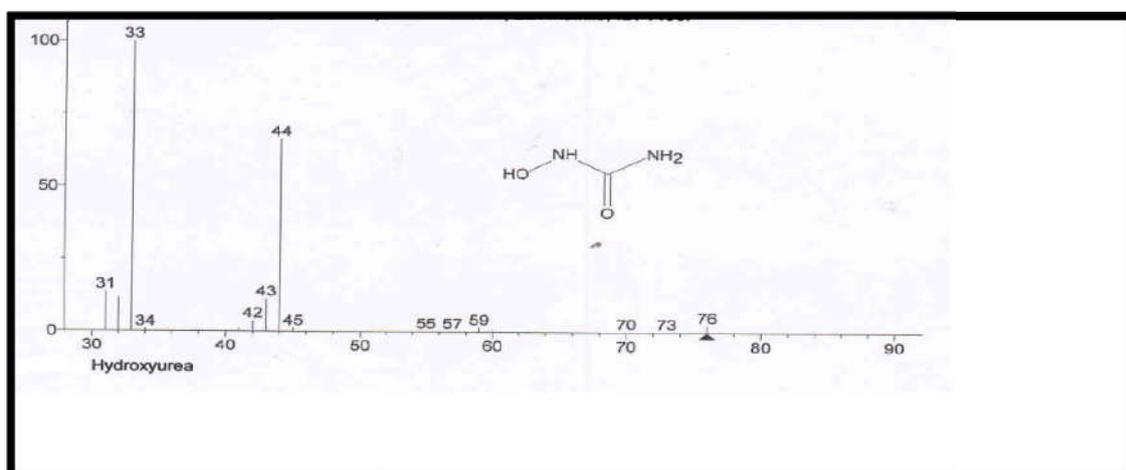


Fig.3 Mass Spectrum for hydroxyl urea

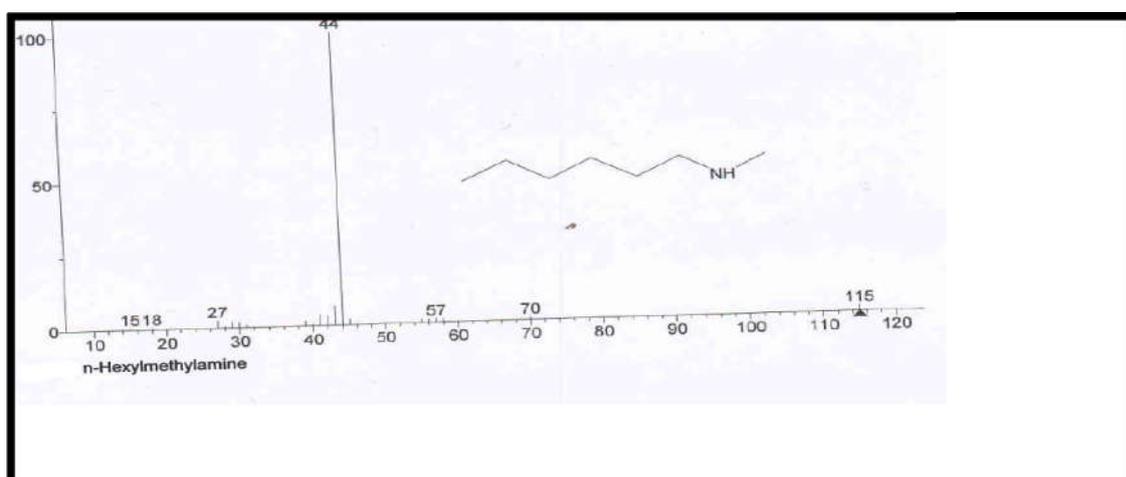


Fig.4 Mass Spectrum for n-hexyl methyl amine

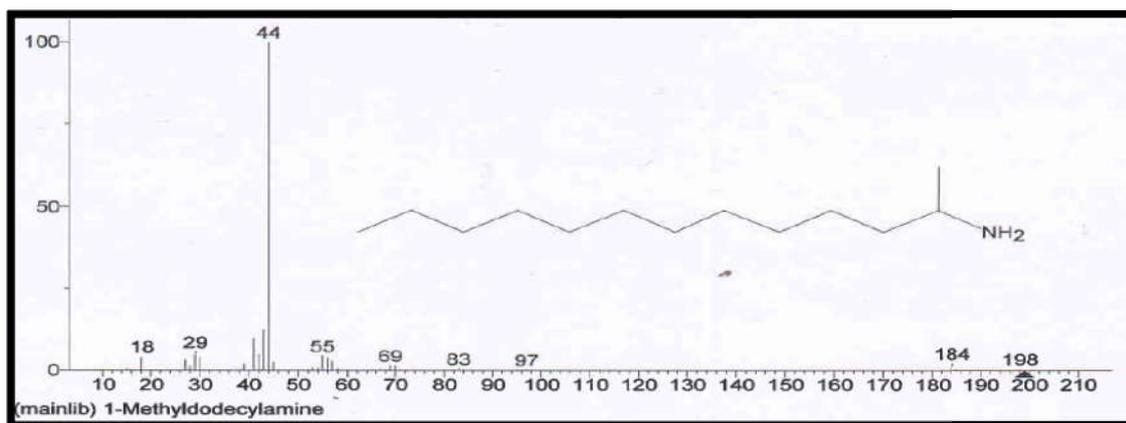


Fig.5 Mass Spectrum for 1-methyl dodecyl amine

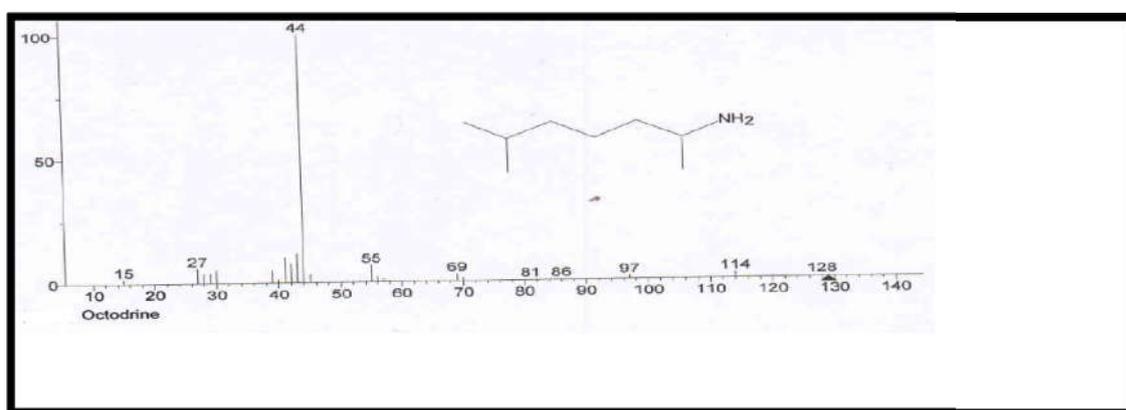


Fig.6 Mass Spectrum for Octadomine

Effect of Oral Administration Alcoholic extract of *Haloxylon salicornicum* on glucose level in alloxan induced diabetic rabbits

The effect of alcoholic extract of *Haloxylon salicornicum* on blood glucose conc. in hyperglycemic rabbits at different times after oral administration, is indicated in table (4). It was found that this extract decreases significantly glucose conc. after 4hrs ($P<0.05$) and a significant decreasing occurred after 6 hrs. ($p<0.01$). The significant decreasing was recorded after 24hrs ($P<0.01$).

Table (4) : Effect of oral administration of alcoholic extract of *Haloxylonsalicornicum* on blood glucose conc. in hyperglycemic rabbits

Groups	N	Blood glucose conc. (mg/dl)				
		0 hrs.	2 hrs.	4 hrs.	6 hrs.	24 hrs.
Control 3ml normal saline	6	328.2± 7.41	330.4± 4.48	317.8± 2.43	312.6± 4.17	283± 4.85
300mg/kg BW (alcoholic extract)	6	339.12 ±2.24	307.1± 4.56	266.94*± 3.28	232**± 3.89	159.47** ±3.43

Blood glucose conc. were represented as mean ± S.E.M.

P* < 0.05, P** < 0.01, N = number of rabbits in each group.

Effect of Oral Administration of the alkaloids extract from *Haloxylon salicornicum* on glucose level in alloxan –induced diabetic rabbits

The means of blood glucose conc. in fasted hyperglycemic rabbits at different times after oral administration of alkaloids extracted from *Haloxylon salicornicum* , are indicated in table (5). A significantly decreasing result of glucose conc. was found after 4 hrs. , 6hrs (P < 0.01) and also a significant decrease was recorded after 24hrs (P<0. 001).

Table (5): Effect of oral administration of alkaloid compounds extracted from *Haloxylon salicornicum* on blood glucose conc. in fasted hyperglycemic rabbits

Groups	N	Blood glucose conc. (mg/dl)				
		0 hrs.	2 hrs.	4 hrs.	6 hrs.	24 hrs.
Control 3ml normal saline	6	341.12± 12.51	332.22± 14.18	358.61± 2.74	316.92± 11.23	299.78± 9.88
300mg/Kg BW (alkaloid compounds)	6	353.21± 7.40	289.85*± 8.86	220.44**± 10.72	182.36**± 6.12	134.31***± 9.10

Blood glucose conc. were represented as mean ± S.E.M.

P* < 0.05, P** < 0.01, P*** < 0.001, N = number of rabbits in each group.

In general Alloxan has been known when injected at different dose causes diabetogenic and induces DNA strand breaks in isolated rat pancreatic islets in vitro to cause Diabetes mellitus (27). This is in accordance with our data, where alloxan injection induced significant increase in blood glucose levels versus the control (non diabetic) group. This is compatible with many of the studies that have been used Alloxan - induced of diabetes in rabbits and rats (28 , 29). Oral administration effect of alcoholic extract and alkaloids of study plant on blood glucose concentrations in hyperglycemic rabbits appeared decreasing effect in hyperglycemic rabbits .The results showed that administration of alcoholic extract group did not any significant difference after 2 hours compared to the control groups, but decreasing blood glucose levels in rabbits induced alloxan after 2 hrs from oral administration with alkaloids extract , because of metabolism of active compounds begins after 2hrs from oral administration of extract (30). This agrees with the findings of previous workers using different medicine plants (31,32). have hypoglycemic activity. These plant extract contain compound like polysaccharides, flavonoids terpenoids and tannins(33,34), steroid , polypeptides and alkaloids (35,36), and these compounds are responsible for the antidiabetic activity. Phyllanthus

amarus leaf extract is reported to contain compounds like lighans, alkaloids, flavonoids, galloatnoids and glycosides (37). The higher blood glucose levels are expected in alloxan induced diabetic mice, since alloxan causes a massive reduction in insulin release, by the destruction of the β - cells of the islets of Langerhans and inducing hyperglycemia (38) reported that there are two possible explanations for the antidiabetic property of aloe barbadensis. It may have exerted its effect by preventing the death of β -cells and it may permit recovery of partially destroyed β -cells, study plant may also have initiated cell proliferation ,may be the hypoglycemic effect come back to active nitrogen compounds in activities of β -cells in pancreas.

CONCLUSIONS

Both alcoholic extract *and alkaloids extracted Haloxylonsalicornicum* have potential hypoglycaemic effect in hyperglycaemic induced rabbits

استخلاص ، عزل وتشخيص بعض المركبات القلويدية من النبات الطبي العراقي *Haloxylon salicornicum* ودراسة تأثيرها المخفض على مستوى كلوكوز الدم في الأرانب المصابة بفرط السكر المستحدث بالالوكسان

جمال حربي حسين

قسم الكيمياء ، كلية العلوم ، جامعة ذي قار ، ذي قار ، العراق.

الخلاصة

أجريت هذه الدراسة لتحديد وتفصي عمل التأثير المخفض لمستخلصات الكحولية والقلويدية المعزولة من النبات الطبي العراقي *Haloxylon salicornicum* لمستويات سكر الدم في الأرانب المستحدث بها فرط السكر بالالوكسان (١٥٠ ملغم / كغم من جسم الحيوان). تم تحضير المستخلصات من نبات قيد الدراسة ، المستخلص الكحولي وعزل عائلة كيميائية فعالة (القلويدات). أجريت اختبارات نوعية أولية لجميع المستخلصات المحضرة ثم تم تشخيص المركبات الكيميائية الفعالة في مستخلص القلويد (**Acetic acid**) (amino carbonyl);

4-fluoro histamine ; hydroxyl urea; n-hexyl methyl amine ; 1-methyl dodecyl amine ; Octadomine بواسطة تقانات TLC و GC-MS ، ثم درس التأثير المخفض لمستويات كلوكوز الدم في الأرانب المصابة بفرط السكر (المحدث بواسطة الالوكسان) لمعرفة فعالية المستخلصات في

تخفيض سكر الدم في هذه الأرانب، إذ أشارت النتائج أن جميع المستخلصات قد أظهرت تخفيضا جيدا لمستويات الكلوكوز، أظهر مستخلص القلويدات المعزولة من *Haloxylon salicornicum* فعالية عالية جدا في تخفيض مستويات سكر الكلوكوز في دم الأرانب . إذ وجد انخفاض معنوي عند ($P<0.05$) في الساعة الثانية بعد التجريع وانخفاضا معنويا ($P<0.01$) في الساعة الرابعة و السادسة وانخفاضا معنويا عاليا وملحوظا ($P<0.001$) في الساعة الرابعة والعشرين في الأرانب المصابة بفرط السكر .

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