

# Bioactivity Guided Isolation, Characterization & Pharmacological Evaluation of $\alpha$ -amyrin from Chloroform Extract of *Morinda pubescens* in STZ induced Diabetic Rats

\*Oday Sajjad Alsawad<sup>1</sup>, Zainab A. Al-Mayyahi<sup>2</sup>, Nameer A. Khudhair<sup>3</sup> Laith Hossein Jawid<sup>4</sup>

<sup>1</sup>Department of Pharmaceutics/ College of pharmacy/ University of basrah, Iraq.

<sup>2</sup>Department of medicine, college of medicine, University of basrah, Iraq.

<sup>3</sup>Department of Veterinary Hygiene, college of Veterinary medicine, University of basrah, Iraq.

<sup>4</sup>Karnataka college of pharmacy, Rajive Gandhi University of health sciences. India.

\*Corresponding author: Dr. Oday Sajjad Alsawad

Department of Pharmaceutics/ College of pharmacy/ University of basrah, Iraq

Uday\_sajad@yahoo.com, +964 770 904 7565

## ABSTRACT

**AIM:** This research has been designed for isolation of  $\alpha$ -amyrin by chloroform extraction from *Morindapubescens* through characterization and pharmacological evaluation for this extraction and its effect on specific biochemical factors in STZ induced diabetic rats.

**MATERIAL & METHODS:** For the isolation of compounds, chloroform and methanol was used as a solvent system. Firstly 100% chloroform was passed through column and then increasing quantity of methanol was used in different ratio (90:10, 80:20, 70:30, 60:40 and finally 50:50). All the fractions were evaluated for antidiabetic activity (data not shown here) and fraction 3 showed a single spot in TLC and it was characterized by spectral techniques.

**RESULTS:** The compound was determined to be  $\alpha$ -amyrin. The anti-diabetic activity with various biochemical markers was accessed in STZ induced diabetic rats. Plasma glucose levels increased significantly ( $P < 0.01$ ) in diabetic rats other than the normal rats.  $\alpha$ -amyrin was extremely meaningful ( $p < 0.001$ ) and marginally important ( $p < 0.01$ ) in lipid profile relative to diabetic group at dose ranges of 5 mg/kg and 10 mg/kg. The effect of  $\alpha$ -amyrin on different lipids as well as raised HDL levels relative to diabetic animals was highly important. The effect of  $\alpha$ -amyrin at 5mg/kg and 10mg/kg in contrast to diabetic groups was extremely important ( $p < 0.001$ ) and medially important ( $p < 0.01$ ) in SGPT and SGOT.

**CONCLUSION:**  $\alpha$ -amyrin extraction by chloroform from *Morindapubescens* plant was found to have antilipidemic and antidiabetic effect through reduce the deleterious effect of diabetes in laboratory rat.

**Keywords:** Bioactivity Guided Isolation,  $\alpha$ -amyrin, *Morindapubescens*, Chloroform Extract, STZ induced Diabetic Rats

## Correspondence:

Oday Sajjad Alsawad

Department of Pharmaceutics/ College of pharmacy/ University of basrah, Iraq

Email: Uday\_sajad@yahoo.com,

Tel: +964 770 904 7565

## INTRODUCTION

Diabetes mellitus is a chronic metabolic disease of glucose metabolism with a prevalence rate of 4-5 % or more in most countries (Koyuturk et al, 2005). It is estimated that more than 180 million peoples are affected by diabetes worldwide. (WHO, 2008) and this number is expected to be doubled by the year 2030 (Khan et al, 2006). Persistent hyperglycemia which results from absolute or relative insulin deficiency (Balkau et al, 2000) and its impact on protein and lipid metabolism results in diabetic micro and macrovascular complications and the subsequent development of neuropathy, retinopathy, coronary, cerebrovascular, and peripheral arterial disease (Lopez Candales, 2001; Li et al, 2004). According to the review of literature, this was found that *Morindapubescens* Linn. is listed in the different medicinal plants utilized generally as diuretic, febrifuge, carminative, laxative, bitter tonic, demulcent, and antibacterial, also for treatment of chronic cystitis, gonorrhoea, acute-chronic inflammatory conditions.

Previous literature review revealed the chloroform extract antidiabetic activity in STZ induced diabetic rats, however, without a scientific evidence of anti-hyperglycemic activity or anti-hyperlipidemic activity in diabetic animals of active constituents present in chloroform extract, the key goals of the research is for establishing scientifically the activity of anti-diabetic, anti-hyperlipidemic activity in diabetic animals for more effective, potent phytoconstituents with fewer side effects as compared to current synthetic drugs (Khan et al., 2006).

## MATERIAL & METHODS

### Procurement and Identification of the plant leaves

*Morindapubescens* leaves have been gathered in July from the Mandsaur area, India, outside the greenhouse, which reveals a raw green color. In the month of July, which displays a rugged surface green color. The plant leaves were carefully cleaned, dried in shade, finely powdered and utilized for extraction techniques using tap water.

### Successive extraction methods

The correctly identified plant leaves is dried in shade at room temperature & after 4-5 days, it is formed into powder by mixing grinder. These should be extracted with different solvent in order to their increasing polarity to get the correct and dependable retention factor. 100gm Powdered drug has been weighed and in Soxhlet it was packed. After that the drug has been extracted continuously by using petroleum for near about 72 hours. Placing a drop from the thimble on a filter paper give any oily spot has ensured total defatting. The extract was dried in air to remove traces of petroleum ether. The dried mark was treated by chloroform.

The percent Yield of the chloroform of *Morindapubescens* were calculated. Chloroform extract was dried and subjected to phytochemical screening (Kokate 1996; Khandelwal 2006)

### Evaluation of Anti-diabetic activity

### Experimental Animals

## Bioactivity Guided Isolation, Characterization & Pharmacological Evaluation of $\alpha$ -amyrin from Chloroform Extract of *Morinda pubescens* in STZ induced Diabetic Rats

Thirty of wister albino rats of Wistar Albino rats of either sex have been used for the entire study and their weight range between 150gm to 200gm. Rats were procured from the CPCSEA approved vendor New Delhi. They have been kept under proper research facility circumstances at  $25\pm 2^{\circ}\text{C}$ , a typical 12-hour light-dark cycle was utilized for the examination. Business pellet diet (MFD, by Nav Maharashtra Chakan Oil Mills Ltd., New Delhi, India), have been administered free access with water along the study.

### Bioactivity guided isolation of constituents from chloroform extract

Silica gel of 50gm has been mixed with extract of 20gm and a very small amount of a proper solvent. The mixture has been triturated in a mortar till a dry free, homogenous flowing mixture has been found. For the isolation of compounds, chloroform and methanol was used as a solvent system. Firstly 100% chloroform was passed through column, and then increasing quantity of methanol was used in different ratio (90:10, 80:20, 70:30, 60:40 and finally 50:50). At 20 drops per minute rate, elute has been gathered and 25 ml was about every fraction, a total of 185 fractions were collected. All the collected fractions have been simultaneously monitored on a TLC plate utilizing chloroform: methanol (84:16) as solvent system. The fractions showing same TLC pattern were merged and four fractions (F1-F4) were finally obtained. Percentage yield of collected elutes were measured in reverence to the total weight of the fraction. All the fractions were evaluated for anti-diabetic activity (data not shown here) and fraction 3 showed a single spot in TLC, it was characterized by spectral techniques.

### Spectral analysis

MS (Mass Spectroscopy), FTIR (Fourier Transform Infrared Spectroscopy), and NMR (Nuclear Magnetic Resonance) are most commonly used instruments for the structural elucidation of natural products. The Shimadzu (Japan) 8400 S FT-IR spectrometer model with potassium bromide pellets ( $\text{Amax}$  in  $\text{cm}^{-1}$ ) was used for the registration of infrared (IR) spectra.  $^1\text{H}$  NMR spectra were reported as internal standard utilizing deuterated-chloroform ( $\text{Me}_4\text{Si}$ ) or deuterated tetramethylsulfoxide containing tetramethylsilane ( $\text{Me}_4\text{Si}$ ) on Bruker multinuclear FT NMR spectrometer Model AV-400, 400MHz. Images s (singlet), d (doublet), t (trio), m (multiplet), and q (group of four) show turn multiplicity. On the TOF MS ES+ mass spectrometer, the MASS range has been registered. Cylinder layer chromatography (TLC) has proven the immaculate essence of mixtures. For TLC (E. Merck), the pre-coated 60F-254-aluminium silica gel (20 cm x 20 cm with thickness of 250  $\mu\text{m}$ ) was used. Iodine was used for TLC plate construction.

### Acute Toxicity Studies

Intense oral poisonousness test was done by the OECD rule No. 423. Wistar Albino Rats have been saved for the time being fasting before drug organization. An aggregate of 3 animals have been utilized that got a solitary oral portion in 50mg/kg, body weight. For a time of 24 hr the animals have been watched for the adjustments in conduct, touchiness responses and so forth Mortality, assuming any, was resolved over a time of about fourteen days. Henceforth in our examinations we chose 1/10, 1/fifth portion for example 5 and 10 mg/kg portion (OECD, 2001).

### Preparation of Doses

Doses for the animals of different groups according to 5 and 10 mg/kg of the isolated compound body weight were calculated. Tween 80 solution having concentration 1% w/v was used to suspend the fixed highest dose of isolated compound.

### Anti-diabetic activity in Streptozotocin (STZ) induced diabetic rats

For the diabetes induction in experimental rats, normal fresh, healthy rats were taken and kept on fasting for 18 hours. Diabetes has been induced by Streptozotocin single dose (Sigma, St. Louis, Mo, USA), in a 40-mg/kg dose. The STZ solution has been ready in citrate buffer (pH 4.5) and managed by intraperitoneal injection, rats had free access for water as well as food. After 3 days with the glucometer, diabetes was measured by estimating the glucose level in blood. The glucometer is focused on the oxidation mechanism of glucose. Rats with more than 250 mg/dl glucose level in blood have been chosen for further analysis (Guruvayoorappan & Sudha, 2008).

### Experimental Design

For assessing the isolated phytoconstituents anti-diabetic activity, total five groups of rats were made every group contains 6 animals. Group I acted as normal control and treated by 0.9% NaCl. Group II was considered as diabetic control and diabetes were induced by STZ in 40 mg/kg dose. Group III & IV served as  $\alpha$ -amyrin treated in 5 & 10 mg/kg dose. Glibenclamide was administered in 5-mg/kg doses to animals of Group V. The  $\alpha$ -amyrin as well as reference drug has been normally managed at two-dose level for 21 days from starting diabetes day.

### Evaluation of Biochemical Parameters

#### Determination of plasma glucose

Glucose content was determined by the technique for Trinder (1969) utilizing an indicative Kit (Sigma Diagnostics Pvt. Ltd). 0.01 mL of plasma standard and deionized water were taken in three separate cylinders; 1 mL of the chemical reagent was added to each cylinder, blended. The temperature was maintained for 15 min at  $37^{\circ}\text{C}$ . The shading created has been perused at 510 nm against a reagent clear. Mg/dL units measure the characteristics of plasma.

#### Determination of Serum total cholesterol (TC)

This particular process has been utilized to determine the cholesterol level in the blood.

Test (T): serum (0.02 ml), plant extract (2.00 ml) were taken.

Standard sample (S): 2.00 ml along with 0.02 ml isolate sample, although for reasonable sample.

(B): DW 0.02 ml and isolate sample 2.00 ml. The mixture has been well mixed at  $20^{\circ}\text{C}$ - $25^{\circ}\text{C}$  for 10 min or at  $37^{\circ}\text{C}$  for 5 min. Absorbance has been tested at 505/670 nm versus blank (Guruvayoorappan and Sudha, 2008).

#### Determination of serum triglycerides (TG)

The (GPO-PAP) technique has been utilized to quantify the lipid oils in the blood. A fixed amount of 0.01 ml serum has been taken in a test tube.

Test (T) where a 1 ml response has been taken. A standard 0.01 ml has been taken in another test tube (S) and a response of 1 ml was read. The mixture has been mixed properly at  $20^{\circ}\text{C}$  -  $25^{\circ}\text{C}$  for 10 minutes. Absorbance versus the reagent was studied at 505 (500-540 nm) (Guruvayoorappan and Sudha, 2008).

#### Determination of HDL-cholesterol

The (CHOD-PAP) technique has been utilized for determining the serum HDL cholesterol level (Henry, 1974). For this 2 ml has been taken from serum in a test tube as well as added precipitation reagent (0.5 ml). The

## Bioactivity Guided Isolation, Characterization & Pharmacological Evaluation of $\alpha$ -amyrin from Chloroform Extract of *Morinda pubescens* in STZ induced Diabetic Rats

blend has been shaken for 10min at 15°C - 25°C then centrifuging for 15min at 4000rpm. The unquestionable supernatant has been tested to validate HDL-C after 2 hours of centrifugation. 1 ml of the supernatant has been extracted in a (T) test tube and 1 ml has been applied to it of the reaction content. A 1ml response substance (B) has been mixed in a test tube, which was 0.1ml DW. DW 0.1ml has been taken in a test tube, a 1ml response material (B) has been fused. The blends have been blended completely, shaking for 10min at 15-25°C, then evaluated the sample absorbance versus reagent clear at 546nm (Guruvayoorappan and Sudha, 2008).

### Determination of LDL cholesterol

LDL cholesterol was estimated by using Friedwald's (1972) formula as follows:

$$\text{LDL in mg \%} = \text{total cholesterol} - \text{HDL-C} - \text{Triglyceride}$$

5

### Determination of VLDL cholesterol

VLDL cholesterol was estimated by using following formula

$$\text{VLDL in mg \%} = \frac{\text{Triglyceride}}{5}$$

5

### Membrane Marker Enzymes

Various membrane marker enzymes i.e. SGOT "Serum glutamate oxaloacetate transaminase" and SGPT "Serum glutamate pyruvate transaminase" were also analyzed by standard diagnostic kit methods (Guruvayoorappan & Sudha, 2008).

### Statistical analysis

The qualities are communicated in mean  $\pm$  SEM. The outcomes have been examined by utilizing one path investigation of change (ANOVA) trailed through "Dunnet's t test" to decide the factual centrality.  $p < 0.05$  has been picked as the criticalness degree. Measurable examination was done utilizing Graph Pad Prism Software 5.0 adaptation.

## RESULTS

### Phytochemical Screening

**Table 1.** Phytochemical test for chloroform extract of leaf of *Morinda pubescens*

S.No.	Test	Chloroform
1.	Carbohydrate ➤ Molish test ➤ Felling test	- -
2.	Glycosides ➤ Bronteger test	-
3.	Alkaloid ➤ Mayer test ➤ Hager test	+ +
4.	Phytosterol + Triterpenoids ➤ Salkowaski test	+
5.	Protein + Amino acid ➤ Biuret test ➤ Ninhydrin test	- -
6.	Phenolic test ➤ Ferric test ➤ Lead acetate test	+ +
7.	Flavonoids ➤ Alkaline test"	-
8.	Saponin ➤ Foam test	-

*Bioactivity Guided Isolation, Characterization & Pharmacological Evaluation of  $\alpha$ -amyrin from Chloroform Extract of Morinda pubescens in STZ induced Diabetic Rats*

9	Mucilage ➤ Iodine test  ➤ Ethanol test	-
---	---	---

"Note:(+) ve indicates positive result, whereas (-) ve indicates negative result"

**Characterization and Identification of compounds**

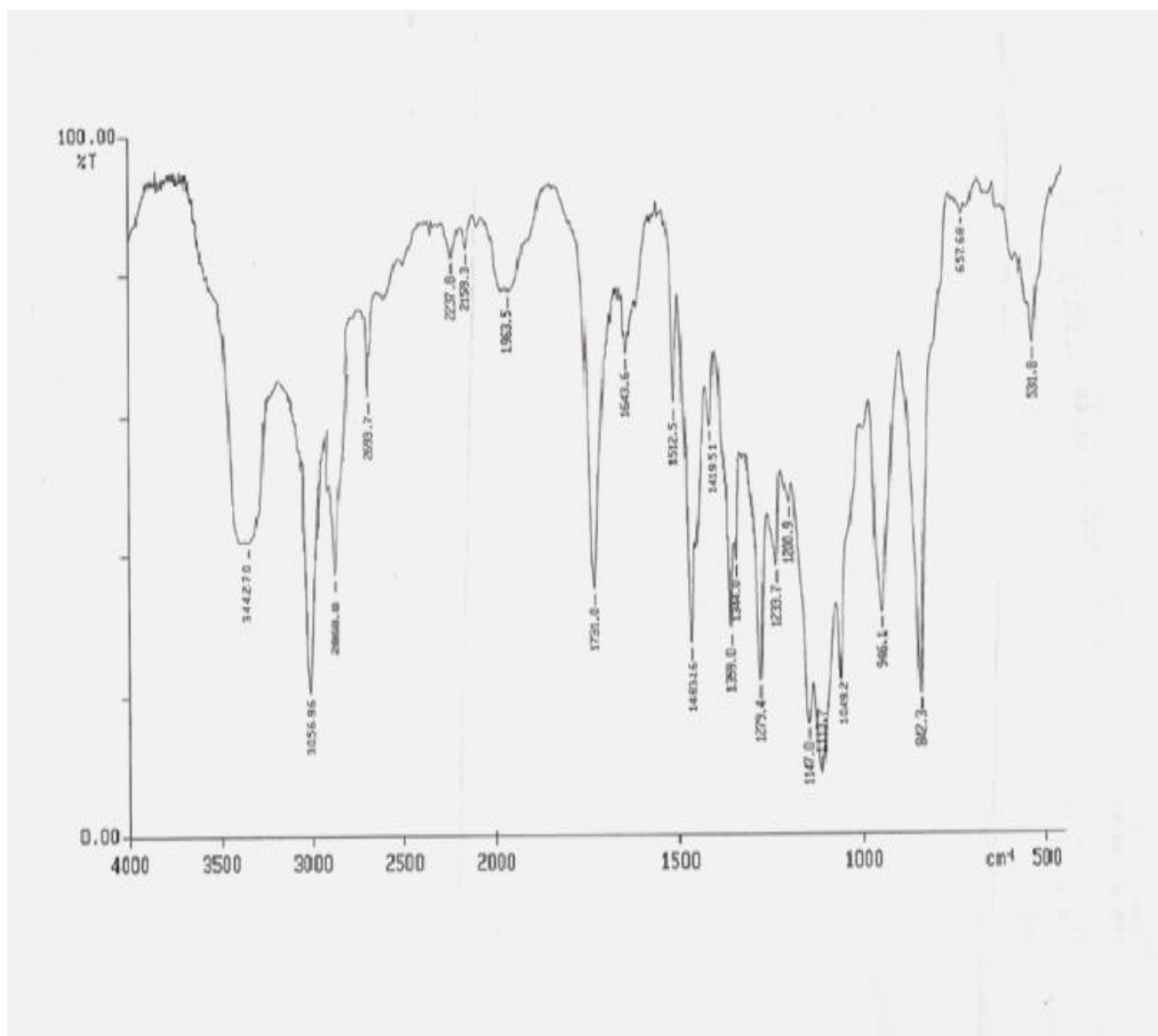
**$\alpha$ -amyrin**

IR (KBr) 3442, 3056, 2860, 2693, 2237, 2159, 1963, 1731, 1643, 1512, 1483, 1419, 1359, 1344, 1279, 1220, 1147, 1049, 946, 842, 657, 537 $\text{cm}^{-1}$

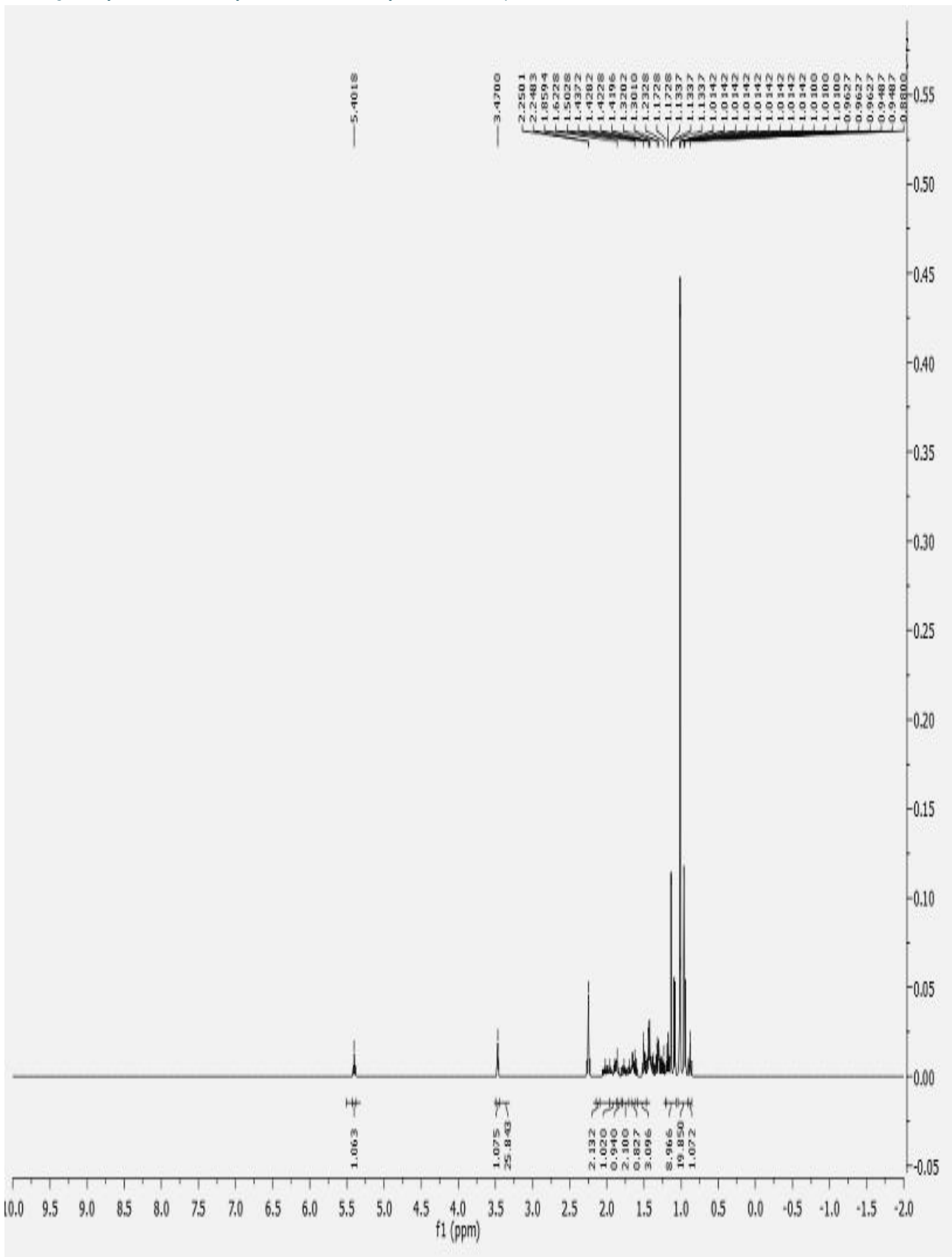
$^1\text{H}$  NMR (400 MHz, Chloroform)  $\delta$  5.40 (s, 7H), 3.47 (s, 7H), 2.80 (s, 6H), 2.23 (s, 7H), 2.02 (s, 5H), "1.96 (dd, J = 9.5, 1.7 Hz, 27H), 1.85 (d, J = 19.4 Hz, 13H), 1.77 (s, 5H),

1.68 (dd, J = 24.5, 5.8 Hz, 1H), 1.74 - 1.21 (m, 122H), 1.21 - 1.16 (m, 18H), 1.03 - 0.99 (m," 41H).

$^{13}\text{C}$  NMR (100 MHz, Common NMR Solvents)  $\delta$  143.02 (s), 125.43 (s), 78.57 (s), 57.72 (s), 54.69(s), 46.52 (s), 42.14 (s), 40.20 (s), 39.67 (s), 38.71 (s), 38.39 (s), 38.04 (d, J = 8.4 Hz), 37.47 (s), 34.06 (s), 32.86 (s), 32.39 (s), 29.16 (s), 27.68 (d, J = 8.7 Hz), 25.88 (s), 24.13 (s), 23.89- 23.67 (m), 23.36 (s), 19.61 (s), 18.79 (s), 18.02 (d, J = 16.2 Hz), 17.05 (s).



"Figure 1. IR Spectra of  $\alpha$ -amyrin"



“Figure 2. <sup>1</sup>H-NMR Spectra of  $\alpha$ -myrin”

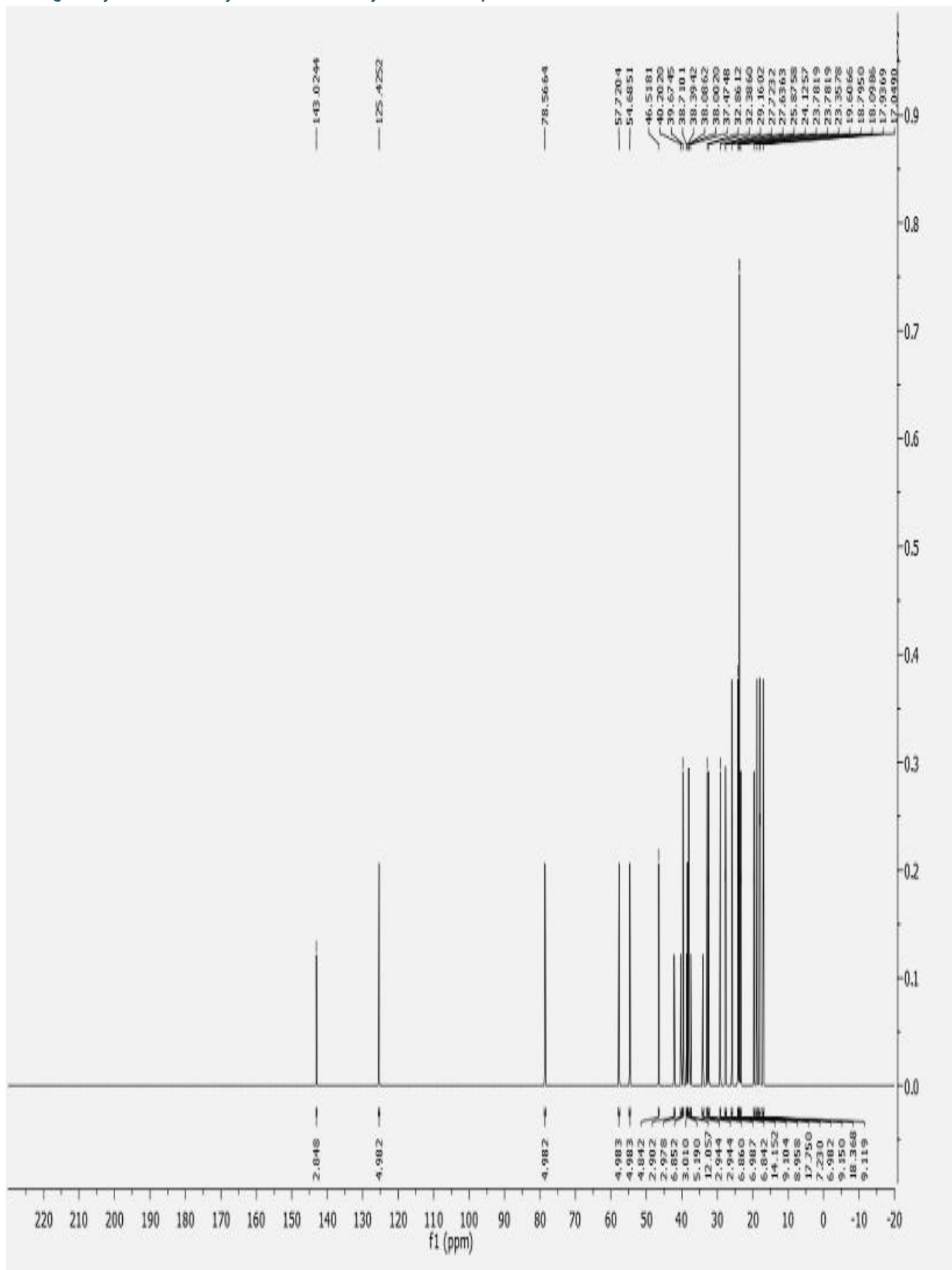
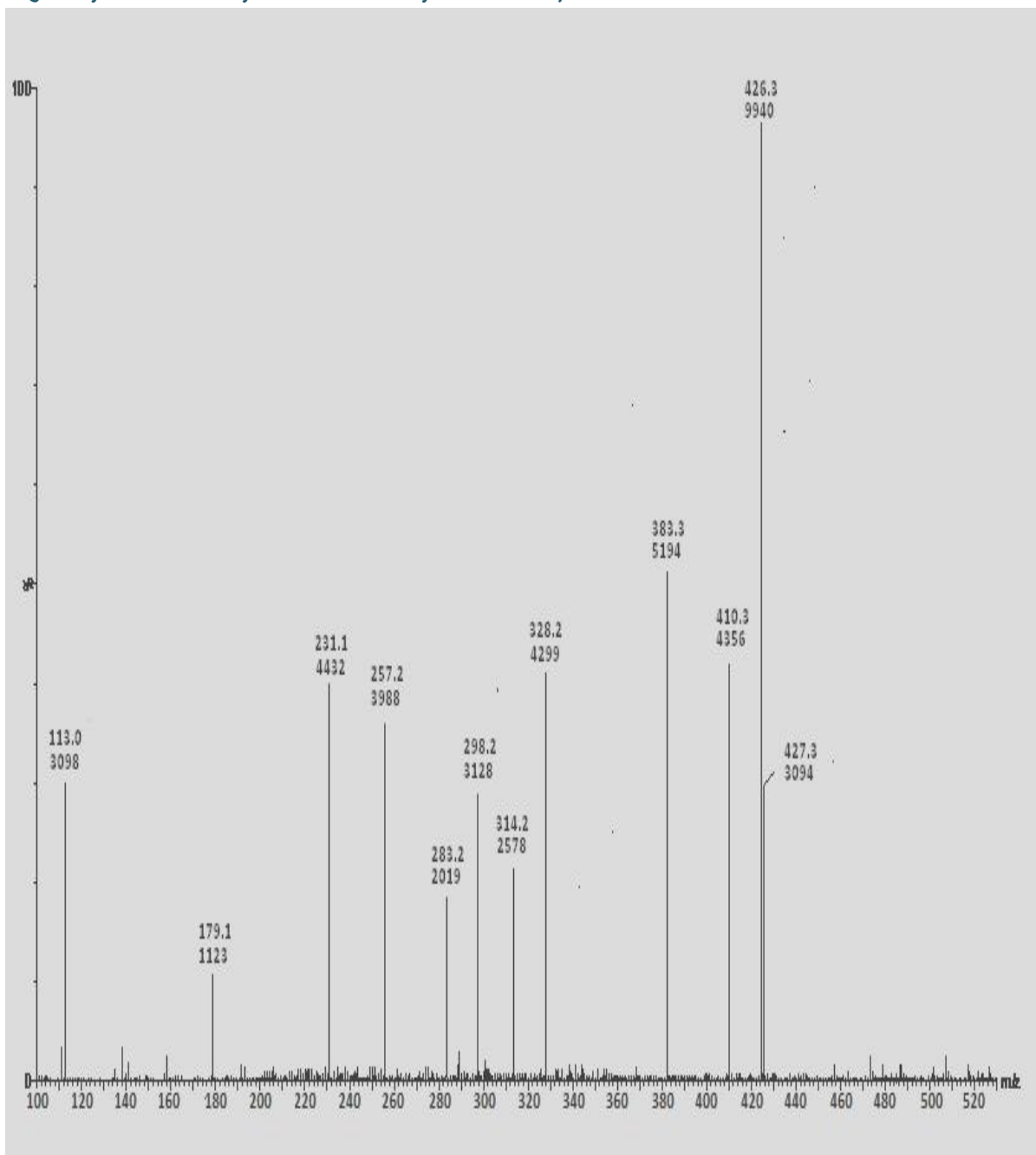


Figure 3.  $^{13}\text{C}$ -NMR Spectra of  $\alpha$ -amyrin



“Figure 4. Mass Spectra of  $\alpha$ -amyrin”

In  $\alpha$ -amyrin, IR absorption bands have been showed at  $3442\text{ cm}^{-1}$  indicating the existence of hydroxyl group,  $3056\text{ cm}^{-1}$  (C-H str. in  $\text{CH}_3$ ),  $2860\text{ cm}^{-1}$  (C-H str. in  $\text{CH}_2$ ),  $1731\text{ cm}^{-1}$  (C=O str.),  $1483\text{ cm}^{-1}$  (C-H def. in  $\text{CH}_3$ ),  $1359\text{ cm}^{-1}$  (C-H deformation in gem dimethyl),  $842\text{ cm}^{-1}$  (=C-H out plane bending). The  $^1\text{H}$ -NMR spectrum presents that H-2 proton appeared at  $\delta$  3.47 as a multiplet and H-13 olefinic proton shows a singlet at  $\delta$  5.40". Saha *et al.* mentioned that 8 methyl protons appeared as multiplet and singlet at  $\delta$  0.88, 0.88,  $\delta$  0.94,  $\delta$  0.96,  $\delta$  1.01,  $\delta$  1.02,  $\delta$  1.13 that have been same with  $\alpha$ -amyrene. The  $^{13}\text{C}$ -NMR demonstrated recognizable signals at 125.43ppm and 143.02 ppm that corresponds to double bond at C-13 along with C-12. At 78.56 ppm,  $\delta$  value is because of C-2  $\beta$ -hydroxyl group. The peaks also presented that the isolated compound had 4 -CH groups, 10- $\text{CH}_2$  group along with 8 methyl group. The outcomes have been compared

with the present literature review and established the  $\alpha$ -amyrin existence.

#### Acute Toxicity Studies

At 50 mg/kg higher doses of Albino Wistar rats no toxic effects have been detected. Thus, an optimal dosage / therapeutic dose was chosen for 1/10th. For activity of anti-diabetic, the cut-off value 5 and 1/5 double dose 10 mg / kg was chosen.

#### Antidiabetic Activity of $\alpha$ -amyrin

##### Effect of $\alpha$ -amyrin on plasma glucose level

Plasma glucose levels seen in Table No.2 in experimental as well as normal rats. In diabetic rats, the amount of plasma glucose changes was large ( $P < 0.01$ ) than the normal rats. Oral doses of  $\alpha$ -amyrin to 10 mg / kg diabetic rats ( $P < 0.001$ ) decreased extremely significantly as compared to rats with diabetic control. 5mg/kg Oral dose for rats with diabetic control decreased moderately

*Bioactivity Guided Isolation, Characterization & Pharmacological Evaluation of  $\alpha$ -amyrin from Chloroform Extract of Morinda pubescens in STZ induced Diabetic Rats*

significantly ( $P < 0.01$ ) other than rats with diabetic control. Plasma glucose reduced moderately suggestively ( $P < 0.01$ ).

**Table 2.** Effect of  $\alpha$ -amyrin on glucose level in streptozotocin induced diabetic rats

Group No	Group	Blood Sugar level (BGL)				
		Long Term Study (Days)				
		Before inducing Diabetes	3	7	14	21
I	Normal control	80.3 ± 2.46	83.3 ± 0.17	82.3 ± 1.7	83.6 ± 1.57	82.11 ± 1.18
II	Diabetic control	82.4 ± 1.81	241.7 ± 1.89	272.8 ± 1.43***	278.3 ± 2.07***	290.1 ± 1.24***
III	$\alpha$ -amyrin treatment (5 mg/kg)	84.27 ± 1.14	240.4 ± 3.25	210.5 ± 3.22***	198.8 ± 2.38***	189.2 ± 2.23***
IV	$\alpha$ -amyrin treatment (10 mg/kg)	87.78 ± 1.29	240.6 ± 2.27	196.3 ± 3.42***	185.6 ± 2.42***	171.3 ± 3.52***
V	Glibenclamide (5 mg/kg)	83.25 ± 0.97	241.8 ± 2.54	197.4 ± 3.23**	167.3 ± 3.44***	162.8 ± 3.21***

*Where- \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared with diabetic control vs treated groups*

**Effect of  $\alpha$ -amyrin on Different Lipid Level**

Diabetic rats were not treated with substantial VLDL-Cholesterol, elevated LDL-Cholesterol, hypertriglyceridemia, and hypercholesterolemia, and HDL Cholesterol reduced in contrast to normal group.  $\alpha$ -amyrin effect on lipid profiles was very positive against hyperlipidemia.  $\alpha$ -amyrin was extremely meaningful

( $p < 0.001$ ) and marginally meaningful ( $p < 0.01$ ) in lipid profile relative to diabetic profile at dose ranges of 5 and 10 mg/kg. The effect of  $\alpha$ -amyrin on different lipids was also very important and HDL was increasing compared with diabetic animals.

**Table 2.** Effect of  $\alpha$ -amyrin on different lipid level in diabetic rats

S. No.	Groups	Parameters				
		Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	HDL-C (mg/dl)
1	Normal Control	76.22 ± 2.33	77.15 ± 2.43	16.53 ± 2.44	17.24 ± 2.56	51.42 ± 2.35
2	Diabetic Control	109.54 ± 3.64	105.26 ± 2.22	53.22 ± 2.93	24.56 ± 1.12	38.14 ± 1.33
5	Diabetic Control + $\alpha$ -amyrin (5 mg/kg)	90.21 ± 2.16*	89.42 ± 2.27*	31.24 ± 2.84***	20.26 ± 1.14*	41.31 ± 1.46**
6	Diabetic Control + $\alpha$ -amyrin (10 mg/kg)	78.21 ± 2.54**	79.37 ± 2.55***	23.28 ± 2.54***	18.41 ± 1.45***	46.28 ± 1.51***
7	Glibenclamide (5 mg/kg)	77.24 ± 2.16***	78.48 ± 2.26***	18.36 ± 2.42***	17.32 ± 1.56***	48.41 ± 3.44***

*Where- \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared with diabetic control vs treated groups*

**Effect of  $\alpha$ -amyrin on SGOT and SGPT level**

Serum biomarkers such as SGPT and SGOT increased significantly in diseased rats than normal rats. A significant difference has been found in the diabetic groups for SGOT and SGPT after the test drugs and standard treatments.  $\alpha$ -amyrin at the 5 mg/kg and

10 mg/kg dose showed very important ( $p < 0.001$ ) and moderately significant effect ( $p < 0.01$ ) on SGOT and SGPT than the diabetic group.  $\alpha$ -amyrin effect on the SGPT as well as SGOT level in contrast to diabetic animals was also important.

**Table 3.** Effect of  $\alpha$ -amyrin on SGOT and SGPT level in diabetic rats

S. No.	Groups	Parameters	
		SGOT (IU/L)	SGPT (IU/L)
1	Normal Control	233.12 ± 2.54	36.26 ± 3.22
2	Diabetic Control	297.15 ± 3.33**	76.31 ± 3.36**
3	Diabetic Control + $\alpha$ -amyrin (5 mg/kg)	281.33 ± 3.34**	62.21 ± 3.27**
4	Diabetic Control + $\alpha$ -amyrin (10 mg/kg)	251.42 ± 2.86***	44.41 ± 3.53**
5	Glibenclamide (5 mg/kg)	238.43 ± 2.63***	41.73 ± 3.96**

*Where- \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared with diabetic control vs treated groups*



## DISCUSSION

Increased production of radicals is a vital reason for tissue pressure and injury. The free radicals extraordinary enduring damage to tissue structures results in an unending change in the nuclear base of starches, lipids, proteins and even nucleic acid destruction. In diabetes, the degree of free radicals was accounted for to increment in alloxan and streptozocin treated rodents. A raised degree of free radicals was identified in a few tissues including the kidneys (Shabeeret *et al.*, 2009). Cell harms will thus, bring about raised creation of ROS "Receptive Oxygen Species". Elevated ROS levels were found to assume a role in the NIDDM pathogenesis (Al-Qattan *et al.*, 2008).

It's well known that insulin which is the treatment of choice for diabetes have many side effect like insulin hypersensitivity, lipodystrophy, lipoatrophy, insulin antibodies, modified metabolic control, autoimmune and other late complexities like morphological changes in kidneys and extreme vascular changes. Likewise, oral hypoglycemic medications have many side effects, for example, heaving, cholestatic jaundice, aplastic, hemolytic anemia's, and dermatological side effect etc. (Mallick *et al.*, 2007; Pepato *et al.*, 2005).

In this investigation,  $\alpha$ -amyrin at the portion of 50 mg/kg listed neither noticeable indications of harmfulness nor mortality and perceptions didn't bring up any evidences of substance related poisonousness. The no-watched unfavorable impact level was seen at the portion of 50 mg/kg. The poisonousness examines was controlled by OECD rules 423. Based on the LD50 esteem, 1/fifth and 1/tenth (5 and 10mg/kg) of its worth has been picked for pharmacological examinations.

The islet  $\beta$ -cells are helpless against damage brought about by oxygen free radicals (Prince and Menon, 1998; Caiet *et al.*, 2005) since the cancer prevention agent guard framework is feeble under diabetic situation. The degrees of cancer prevention agent protection structure are changed in streptozotocin-instigated diabetic rodents that are in acceptable relationship with the current perception. Non protein thiol such as glutathione are the huge basic watches, which check the oxidative weight. Diminished serum glutathione degrees in streptozotocin diabetic rodents that are in reliable with prior reports (Caiet *et al.*, 2005). The watched decline might be because of use of "non-protein thiol through expanded sans oxygen radicals created in hyperglycemic conditions related with diabetes mellitus".  $\alpha$ -amyrin at 5mg/kg and 10mg/kg body weight after treatment of 21 days created a stamped decline in blood glucose levels in STZ models. As the outcomes appeared, antidiabetic impact of  $\alpha$ -amyrin might be because of expanded arrival of insulin from  $\beta$ -cells.

The one and only plant kingdom (Chandiran *et al.*, 2014; Pacific, 1992) is an enormous pool of biologically active material with a specific chemical composition and disease prevention properties. That is why herbal therapies have become more interested as an alternative to allopathic medicines and lately the demand for these medicinal products has grown considerably.  $\alpha$ -amyrin also showed a highly significant effect in glucose tolerance test and antidiabetic effect may be independent of insulin release. STZ-diabetic rodents showed increase in cholesterol and fatty acid levels (Khemani and Sachdewa, 2003), which may add to the unforeseen development as well as enlargement of small and full-scale vascular

ensnarement (Tan *et al.*, 2005). This was understood that hyperlipidemia incited with hyperglycemia is a critical cardiovascular mortality determinant and is associated with diabetes mellitus. Plasma lipoprotein metabolism changes are basic in diabetes and will in general misrepresent any prior propensities towards raised lipid levels (Merzouk, 2004). Since insulin restrains the touchy lipases, the last gets dynamic without insulin. Thus, weakening of hyperglycemia or lipoproteins glycation, receptors and compounds engaged with lipid metabolism may diminish the cardiovascular demise risk in diabetic patients.

The increase in plasma triacylglycerols, cholesterol and LDL-cholesterol ratio in the current investigation show unsettling of lipid metabolism and expanded occurrence of cardiovascular damage in diabetic rodents. Then again, "glucagon and different hormones upgrade lipolysis. The stamped hyperlipidemia, which describes the diabetic state, may, thusly, be viewed as a result of uninhibited lipolytic hormones activities on the fat" stores (Ramesh and Pugalendi, 2005). Analyses on STZ-incited diabetes in test animals have showed that an extension in circulatory VLDL and their connected fatty acids are a direct result of lacking room of these particles from the stream (Suresh Babu, 1997).

Regularly flowing LDL-C goes through reuptake in the liver by means of explicit receptors that gets cleared through the dissemination (Lusis, 2000). HDL-C is defensive by turning around cholesterol transport, repressing the LDL-C oxidation through killing the atherogenic oxidized LDL-C impacts. The expanded VLDL-C and LDL-C degrees diminish HDL-C as there is a complementary connection between the LDL-C as well as VLDL-C convergence. In diabetic rodents treated with  $\alpha$ -amyrin there is a raise in HDL-C, reduced in VLDL-C along with LDL-C. There is a cozy connection between atherosclerosis event and total cholesterol plasma level and, the capacity of  $\alpha$ -amyrin is reflected in the specific decrease of all cholesterol, the decrease of LDL as well as VLDL segments. This could be advantageous in forestalling atherosclerotic situation, accordingly diminishing the chance of coronary illness. It is consequently vital that the impact of concentrate on plasma HDL plainly shows that the degree of this lipoprotein division expanded with  $\alpha$ -amyrin treatment.

Cholesterol is the main risk factor for CHD "Coronary Heart Diseases". The hypercholesterolemia degree is comparative directly with diabetes. In the current assessment, we have practical more raised cholesterol levels in diabetic rodents' tissues. The extended cholesterol level in tissues could be a result of the reduced HDL-cholesterol level. Hence this achieves decreased cholesterol release from extra hepatic tissues through the HDL cholesterol (Ashok kumar *et al.*, 2005; Mendez and Balderas, 2001). Treatment of  $\alpha$ -amyrin to STZ diabetic rodents normalizes cholesterol levels in plasma by reduced in cholesterol supply from the stomach related parcel, by binding with bile acids in the stomach related framework as well as increase bile acids release.

One of the risk factors for CHD is the mass of fatty compounds. The dyslipidemia's features in type 2 diabetes are elevated fatty acid and reduced HDL

## Bioactivity Guided Isolation, Characterization & Pharmacological Evaluation of $\alpha$ -amyrin from Chloroform Extract of *Morinda pubescens* in STZ induced Diabetic Rats

cholesterol (Lehto et al., 1997). Hypertriglyceridemia in type 2 diabetes is consequence from an extended hepatic VLDL over creation and blocked catabolism of fatty acid rich particles. Both insulin resistance and insulin difficulty are preventing the lipoprotein lipase limits (LPL), which are an essential impulse in the reduction and deprivation of fatty acids. LPL's degradation leads for fasting and post-prandial hypertriglyceridemia. The high plasma fatty acid level has been thought to have an effect on LDL measurements and thickness via a lipid exchange example (Taskinen et al., 1996).

The expanded grouping of free unsaturated fats was seen in liver and kidney of diabetic rodents and this might be because of lipid collapse and this may basis over NADPH creation that brings about the actuation of NADPH subordinate microsomal lipid peroxidation.

A few examinations have detailed that the expanded phospholipids levels have been found in the diabetic rodents' tissues. In diabetic rodents, the raised degree of phospholipids might be because of the raised degrees of free unsaturated fats and the total cholesterol (Frayn, 1993). The rebuilding of phospholipids by the organization of  $\alpha$ -amyrin might be because of controlled activation of plasma fatty oils; controlling the tissue metabolism and improving the degree of insulin release and activity apparently interceding cholesterol and phospholipids.

LCAT is a plasma chemical that represents the development of the cholesteryl esters greater part in plasma on the HDL outside (Lima et al., 2004). The diminished LCAT action particularly diminished the degrees of developed HDL particles, which may eventually prompt extreme hindrance of the HDL-C combination just as fatty substances metabolism in diabetic rodents. Reclamation of the lipid profile in  $\alpha$ -amyrin treated diabetic rodents could be the reason for expanded LCAT action.

Hepatic serum biomarkers such as SGPT and SGOT have been assessed on 28<sup>th</sup> day as well as utilized for hepatic damage assessment. Diabetic rodents demonstrated raised degrees of SGOT and SGPT which are known to be marker of hepatic damage in any case,  $\alpha$ -amyrin causes critical decline in level of SGOT and SGPT.

### ACKNOWLEDGMENTS

The researchers thank the deans of the Faculties of Pharmacy, Veterinary Medicine at the University of Basra and the laboratory personnel in these colleges as well as the Karnataka College of Health and Pharmaceutical Sciences (Rajiv Gandhi University in India).

### CONFLICTS OF INTEREST

For anyone, no interest conflicts have been occurred.

### FUNDING OF RESEARCH

The researchers themselves funded the research.

### AUTHOR'S CONTRIBUTIONS

Part of this study has been conducted in the laboratories of the College of veterinary medicine / University of Basra and the laboratories of the Karnataka College of Health and Pharmaceutical Sciences (Rajiv Gandhi University, India). This manuscript was worked on over a period of 9 months with serious and continuous work.

### CONCLUSION

The laboratory work included extracting and isolating the active compound  $\alpha$ -amyrin from the leaves of *Morinda pubescens*, diagnosing it, then performing phytochemical tests by internationally approved analytical methods. After that, biological and pharmacological studies of the active compound  $\alpha$ -amyrin were performed.

### ETHICS

We undertake, after publishing of the manuscript, to resolve any health or ethical problems.

### BIBLIOGRAPHY

1. Koyuturk, M., Ozsoy-Sacan, O., Bolkent, S., Yanardag, R., 2005. Effect of glurenorm on immunohistochemical changes in pancreatic  $\beta$ -cells of rats in experimental diabetes, *Indian Journal of Experimental Biology* 43, 268–271.
2. Khan M S Y, Bano S, Javad K, AsadMueed M., "A comprehensive review on the chemistry and pharmacology of *Corchorus* species- A source of cardiac glycosides, triterpenoids, ionones, flavonoids, coumarins, steroids and some other compounds" *Journal of Scientific & industrial research*, vol. 65 April 2006, pp 283-298.
3. Balkau B, Charles MA, Eschwege E (2000). Epidemiological discourse on new criteria on diabetes. *Molecular endocrinology* 2: 229-234.
4. Lopez Candaless, C.A., 2001. Metabolic syndrome X; a comprehensive review of the pathophysiology and recommended therapy. *Jornal do médico* 32:283-300.
5. Li, W.L.; Zheng, H.C.; Bukuru, J.; and De Kimpe, N.; *J. Ethnopharmacol.* 92, 2004, 1–21.
6. Shabeer J, Srivastava R S, Singh S K, Antidiabetic and antioxidant effect of various fractions of *Phyllanthus simplex* in alloxan diabetic rats. *Journal of Ethnopharmacology* 2009; 124: 34–38.
7. Al-Qattan K, Martha Thomson a, Muslim Ali, Garlic (*Allium sativum*) and ginger (*Zingiber officinale*) attenuate structural nephropathy progression in streptozotocin-induced diabetic rats. *The European e-Journal of Clinical Nutrition and Metabolism* 2008; 3: e62-e71.
8. Mallick C, Chatterjee K, Biswas M G, Ghosh D. Antihyperglycemic effects of separate and composite extract of *Musa paradisiacal* and leaf of *Coccinia indica* in streptozocin induced diabetic male albino rat. *African Journal of Traditional, complementary and alternative medicine* 2007; 4(3): 362-371.
9. Pepato M T, Mori D M, Baviera A M, Harami J B, Vendramini R C, Brunetti I L. Fruit of the jambolan tree (*Eugenia jambolana* Lam.) and experimental diabetes. *Journal of Ethnopharmacology* 2005; 96: 43–48.
10. Prince P S M, Menon V P. Effect of *Syzygium cumini* in plasma antioxidants on alloxan-induced diabetes in rats, *Journal of Clinical Biochemistry and Nutrition* 1998; 25(2): 81–86.
11. Cai L, Wang J, Li Y, Sun X, Wang L, Zhou Z, Kang Y J. Inhibition of superoxide generation and associated nitrosative damage is involved in metallothionein prevention of diabetic cardiomyopathy. *Diabetes* 2005; 54: 1829-1837.
12. Pacifici, R.E.; and Davies, K.J.; *Gerontology.* 37, 1991, 166-180.
13. Chandiran SI, Jayaveera KN, and Shaik K. *Int. J. Res. Pharm. Sci.* 5 (1), 2014, 79-84.

*Bioactivity Guided Isolation, Characterization & Pharmacological Evaluation of  $\alpha$ -amyrin from Chloroform Extract of Morinda pubescens in STZ induced Diabetic Rats*

14. Sachdewa A, Khemani LD. Effect of Hibiscus rosasinensis Linn. ethanol flower extract on blood glucose and lipid profile in streptozotocin induced diabetes in rats. *J. Ethnopharmacol.* 2003; 89: 61-66.
15. Tan BK, Tan CH, Pushparaj PN. Anti-diabetic activity of the semi purified fractions of Averrhoabilimbi in high fat diet fed-Streptozotocin induced diabetic rats. *Life Sci.* 2005; 76: 2827-2839.
16. Merzouk S, Hichami A, Sari A, Madani S, Habane SN, Khan NA. Impaired oxidant/antioxidant status and LDL-fatty acid composition are associated with increased susceptibility to peroxidation of LDL in diabetic patients. *Gen. Physiol. Biophys.* 2004; 23: 387-399.
17. Ramesh B, Pugalendi KV. Antihyperglycaemic effect of umbelliferone in STZ-diabetic rats. *J. Med. Food.* 2006; 9: 562-566.
18. Suresh Babu P, Srinivasan Hypolipidaemic action of Curcumin, the active principle of turmeric (*Curcuma longa*) in streptozotocin induced diabetic rats. *Mol. Cell. Biochem.* 1997; 166: 169-175.
19. Porras-Morfin, V., Meoño-Sánchez, E., Peralta-Moreno, K., Quinto, A. -, Esquite-Montoya, J., & Guerra-Centeno, D. (2018). Relationship between the length of the beak and the sex in keel-billed toucans (*ramphastos sulfuratus*). [Relación entre el largo del pico y el sexo en tucán pico arcoíris (*Ramphastos sulfuratus*)] *Revista Electronica De Veterinaria*, 19(5)
20. Lusic JA. Atherosclerosis. *Nature* 2000; 407: 233-241.
21. Ashokkumar N, Pari L, Manimekalai A, Selvaraju K. Effect of N- benzoyl- D-phenylalanine on streptozotocin induced changes in the lipids and lipoprotein profile in rats. *J. Pharm. Pharmacol.* 2005; 57: 359-366.
22. Mendez JD, Balderas F. Regulation of hyperglycemia and dyslipidemia by exogenous L-arginine in diabetic rats. *Biochimie.* 2001; 83: 453-458.
23. Lehto S, Haffner SM, Pyorala K, Kallio, Laakso M. Dyslipidemia and hyperglycemia predict coronary heart disease events in middle aged patients with NIDDM. *Diabetes.* 1997; 46: 1364-1369.
24. Taskinen MR, Lahdenpera S, Syvanne M. New insights into lipid metabolism in non-insulin dependent diabetes mellitus. *Ann. Med.* 1996; 28: 335-340.
25. Frayn KN. Insulin resistance and lipid metabolism. *Curr. Opin. Lipidol.* 1993; 4: 197-204.
26. Lima VLM, Coelho LCBB, Kennedy JF, Owen JS, Dolphin PJ. Lecithin: cholesterol acyl transferase (LCAT) as a plasma glycoprotein: an overview. *Carbohydrate Polymers.* 2004; 55: 179-191.
27. Guruvayoorappan C, Sudha G. Phytopharmacological evaluation of Byesukar for hypoglycaemic activity and its effect on lipid profile and hepatic enzymes of glucose metabolism in diabetic rats. *Ann. Hepat.* 2008; 7: 358-363.
28. Mukherjee, P.K., 2002. *Quality Control of Herbal Drugs-an Approach to Evaluation of Botanicals*. New Delhi, Business Horizons Pharmaceutical Publishers.
29. Kokate, C.K., 1996, *Practical Pharmacognosy*. Delhi, VallabhPrakashan.
30. Khandelwal, K.R., 2006. *Practical Pharmacognosy*. Pune, NiraliPrakashan.
31. OECD Guidelines 2001. "Guidance document on acute oral toxicity testing" Series on testing and assessment No. 23, Organization for Economic Co-operation and Development, OECD Environment, health and safety publications, Paris Available from: <http://www.Oecd.org/ehs> [accessed 20 March on 2010].