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SYNTHESIS, CHARACTERIZATION, PHARMACEUTICAL POTENTIAL AND OPTICAL PROPERTIES OF 3,3,6,6-TETRAMETHYL-9-PHENYL-3,4,6,7,9,10- HEXAHYDROACRIDINE-1,8(2H,5H)-DIONE DERIVATIVES

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ABSTRACT

The hexahydroacridne derivatives was synthesised and characterised by various techniques. Nuclear Magnetic Resonance (NMR) spectroscopy is a well established technique for providing information about structural diagnosis of organic molecule and mass spectrometry. Optical properties of hexahydroacridine derivatives, has been made by experimentally. Synthezied hexahydroacridine derivatives were screened for antioxidant, anti inflammation, and anti diabetic activity. The compounds were screened for their Antioxidant activity was evaluated by DPPH radical scavenging method and compared with standard ascorbic acid. Anti inflammation activity were examined by albumin denatuation and compared with standard aspine. Antidiabetic activity against α-amylase enzyme and compared with standard drug acarbose.

Keywords: release NMR, Optical ,antioxidant, anti-inflammatory and anti-diabetic agent.

1.Introduction

Acridine is an organic compound and a nitrogen heterocycle, with the formula C13H9N. Acridine is obtained from the high boiling fraction of coal tar. It is also obtained in nature from a plant and marine sources. Acridine undergoes a number of reactions such as nucleophilic addition, electrophilic substitution, oxidation, reduction, reductive alkylation, and photoalkylation. Acridine derivatives have engaged a exclusive position in medicinal chemistry due to their wide range of biological purpose[1] . Acridinediones and their derivatives have a broad range of pharmaceutical activities, including antimicrobial[2] , antimalarial[3] , antitumor[4] , anticancer[5], antibacterial[6] , fungicidal[7] , and DNA binding properties[8] . These derivatives havebeen used in chemotherapy for the action of cancer[9] and the behavior of cardiovascular diseases, such as angina pectoris and hypertension. Acridinediones derivatives also possess photophysical/electrochemical properties[10-12] and are found to be good antimalarial agents, and the analogue compounds are analysed as a potent antimalarial activity[13] . A series of 9-anilinoacridines were investigated for their abilities to inhibit -hematin formation and to form drug hematin complexes . In general, poor relation between inhibition and antimalarial activity was observed, only one compound may exerts its antimalarial activity by inhibiting hemozoin

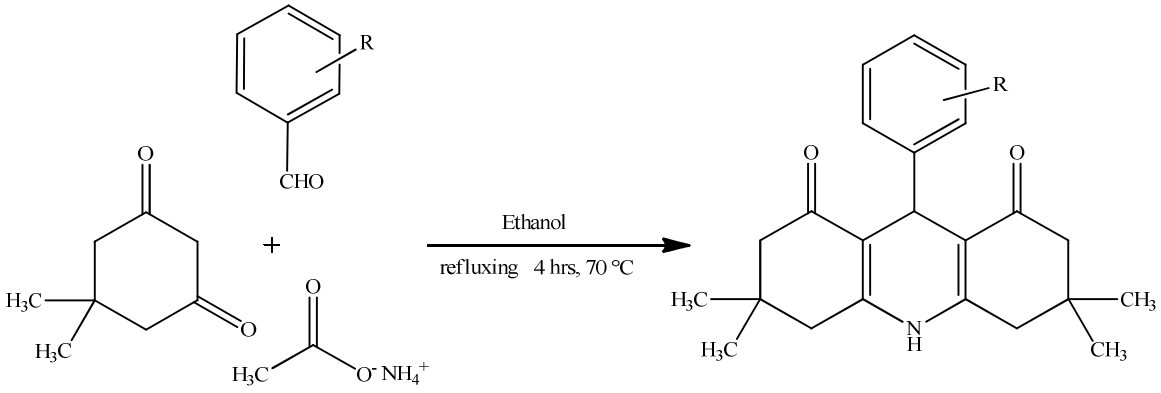
formation.Acridinediones containing a 1,4-DHP (Dihydropyridine) nucleus are used as laser dyes with very high efficiencies of photo initiators [14,15]. Hexahydroacridine-1,8-dione derivatives are also reported to possess important properties such as high fluorescence efficiency [16].In the present paper, we have studied the NMR, UV–Vis spectra and PL of the title molecule.

2.Materials and methods

Materials and measurements Chemical reagents such as dimedone, substituted aldehyde, ammonium acetate were offered by Sigma-aldrich (St.Louis, USA). The UV–Visible spectra are calculated in UV–Vis spectrophotometer Perkin Elmer, Lambda 35 and the correction done in the background owing the polar solvent absorptions.1H and 13C (400 MHz) NMR spectra are documented on Bruker NMR spectrometer using DMSO solvent. A PL spectrum is documented on a Perkin Elmer LS55 fluorescence spectrometer.

2.1 Synthesis of hexahydroacridine derivatives

A mixture of 5,5-dimethylcyclohexane-1,3-dione (2 mmol), substituted aldehyde (1 mmol), ammonium acetate (1 mmol) under refluxing for 4hrs at 70 °C (Scheme 1). The progress of the reaction was followed by TLC. After completion of the reaction, the reaction mixture was cooled, dissolved in acetone and filtered. The product was purified by column chromatography with benzene: ethyl acetate (9:1) as the eluent.



R = 4-Br, (4-OCH3, 3-OH)
Scheme 1

2.2 Anti-Oxidant Studies

DPPH scavenging assay

The ability to scavenging the stable free radical, DPPH was measured as a decrease in absorbance at 517 nm by the method of Mensor et al., (2001). Reagents 2,2-Diphenyl-1-picryl hydrazyl (DPPH)● – 90.25mM in methanol in a dark room. Procedure To a methanolic solution

of DPPH (90.25 mM), an equal volume of ethanolic Rhizome of Cyperus rotundus L (250-1500 µg) was added and made up to 1.0 mL with methanolic DPPH. An equal amount of methanol was added to the control. After 20 min, the absorbance was recorded at 517 nm in a Systronics UV-visible Spectrophotometer. Ascorbic acid was used as standard for comparison. The inhibition of free radicals by DPPH in percentage terms (%) was calculated by using the following equation. % Scavenging = A Control OD - A sample ×100 A blank5 Where A control is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test compound.

2.3 Anti- inflammatory activity:

Inhibition of Albumen Denaturation

Method as prescribed (Sakat et al., 2010) was followed with modifications. The reaction mixture was consisting of test extracts and 1% solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount at 37°C HCl. The sample extracts were incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes after cooling the samples the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug. The experiment was performed in triplicates. Percent inhibition of protein denaturation was calculated as follows: Percent inhibition (%) = (OD of Control- OD of Sample/ OD of Control) X 100.

2.4 Inhibition Of Alpha-Amylase Enzyme

Starch solution (0.1% w/v) was prepared by stirring 0.1 g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of αamylase in 100 ml of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartarate solution and 3,5-di nitro salicylic acid solution 96 mM. The starch solution is added to the both control and plants extract tubes and left to react with α-amylase solution,under alkaline conditions at 25ºC. The reaction was allowed for 3 min. The generation of maltose was quantified by the reduction of 3,5-dinitro salicylic acid to 3-amino-5-nitro salicylic acid. This reaction is detectable at 540 nm (Malik and Singh 1980)

$$\% \text{ Inhibition} = \frac{\text{Control OD - Test OD}}{\text{Control OD}} \times 100$$

3.Results and discussion

3.1 NMR spectrum of hexahydroacridine derivatives

9-(4-bromophenyl)-3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydroacridine-1,8(2H,5H)- dione (1)

0.86 (s, 6H), 1.0 (s,δMolecular formula for C23H26BrNO2: 1H NMR (400 MHz, DMSO): 6H), 1.96 (s, 4H), 2.43 (s, 4H), 4.76 (s, 1H), 7.10 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.4 Hz, 26.92, 29.51, 32.61, 33.27, 40.51, 50.60,δ2H), 9.38 (s, 1H). 13C NMR (400 MHz, DMSO): 111.47, 118.94, 130.37, 130.91, 146.96, 150.04, 194.96. Theoritical value of MS: m/z. 427.11 [M+]

9-(3-hydroxy-4-methoxyphenyl)-3,3,6,6-tetramethyl-3,4,6,7,9,10 hexahydroacridine1,8(2H,5H)-dione - (2)

0.83 (s, 6H), 1.0 (s, 6H),δMolecular formula for C24H29NO4: 1H NMR (400 MHz, DMSO): 2.02 (s, 4H), 2.42 (s, 4H), 3.66 (s, 3H), 4.79 (s, 1H), 6.61 (s, 1H), 6.62 (d, J = 8.0 Hz, 1H), 26.89,δ6.71 (d, J = 12.0 Hz, 1H), 7.05 (s, 1H), 9.32 (s, 1H). 13C NMR (400 MHz, DMSO): 29.57, 32.59, 33.15, 40.52, 50.69, 55.18, 110.77, 111.77, 114.35, 120.42, 129.03, 149.03, 149.91, 159.12, 194.96. Theoritical value of MS: m/z. 395.21 [M+].

3.2 Optical properties of hexahydroacridine derivatives

The absorption spectra of hexahydroacridine derivatives at different concentration are exhibited in (Figure 1). When the concentration increases, certainly the potency of absorption spectra also raises. Therefore by growing the concentration of hexahydroacridine derivatives the potency of emission spectra is raises (Figure 2). From these spectra we conclude all the hexahydroacridine derivatives deliver the outstanding optical properties.

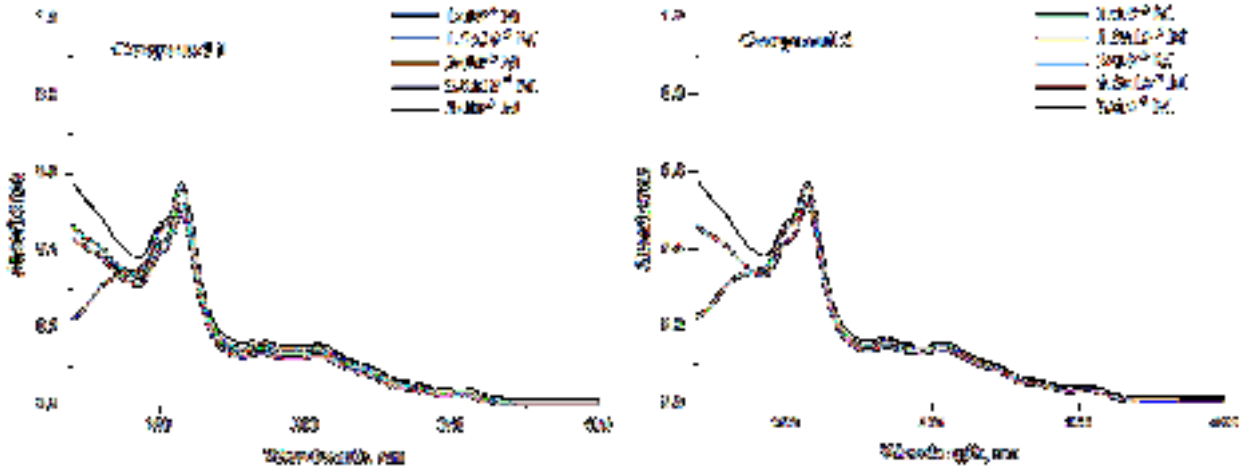


Figure 1

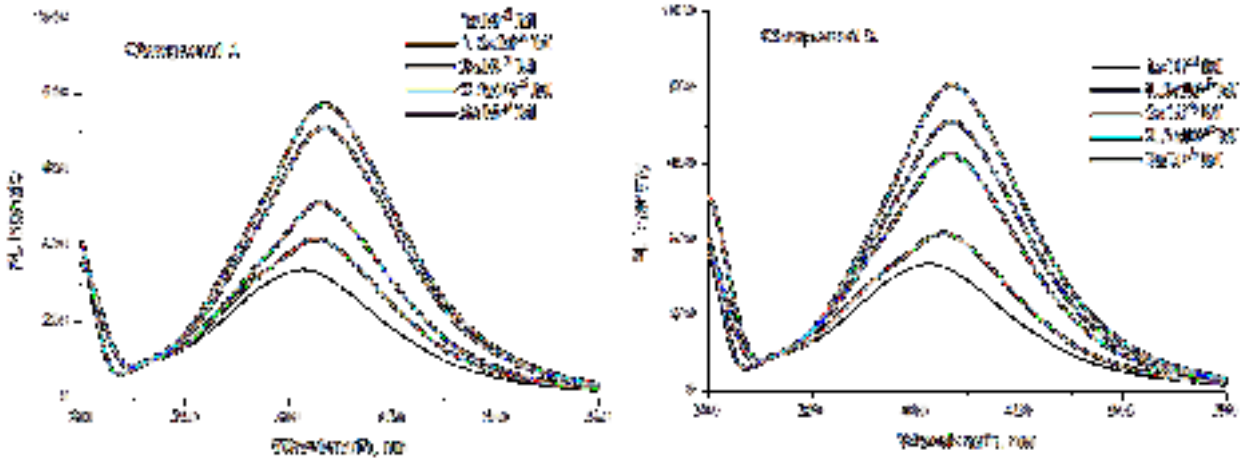


Figure 2

3.3 Anti-Oxidant Studies

DPPH scavenging assay method

There are several methods available to assess the antioxidant activity of compounds. DPPH free radical scavenging assay is an easy, rapid, and sensitive method for the antioxidant screening of hexahydroacridine. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases.

In the present study, the derivatives of hexahydroaciridinehigh DPPH scavenging capacity, which increased with increasing concentration [Table 1 and Figure 3]. It is evident from the data presented in Table, that the sample possesses DPPH assay activity. For the 9-(4-Bromophenyl)-3,3,6,6 tetramethyl-3,4,6Tetramethyl 3,4,6,7,9,10 – hexahydro– 2H, 5H –acridine 1,8 –Dione [GS1C], the result shows the percentage of cytotoxicity for 0.2 mg/ml as 56.8%, 0.4 mg/ml as 68.9% , 0.6 mg/ml as 80.0% , 0.8 mg/ml as 53.6% and 1.0 mg/ml as 100.9%. For the 9-(3-hydroxy 4-methoxy phenyl)-3,3,6,6 tetramethyl- 3,4,6,7,9,10-hexahydro-2H,5H- acridine- 1,8-dione [GS2C], the result shows the percentage of cytotoxicity for 0.2 mg/ml as 21.5%, 0.4 mg/ml as 33.2% , 0.6 mg/ml as 43.9% , 0.8 mg/ml as 53.6% and 1.0 mg/ml as 63.8%. These inhibition values are compared with standard drug of **Ascorbic acid for** for 0.2 mg/ml as 35%, 0.4 mg/ml as 46% , 0.6 mg/ml as 56% , 0.8 mg/ml as 66% and 1.0 mg/ml as 77%

S.No	Test	Concentration of the sample (mg/ml)	% of inhibition of the GS1C	% of inhibition of the GS2C	Ascorbic acid (Standard)

1	DPPH	0.2	56.8	21.5	35.0
2		0.4	68.9	33.2	46.0
3		0.6	80.0	43.9	56.O
4		0.8	93.1	53.6	66.0
5		1.0	100.9	63.8	77.0

Table.1 Anti Oxidant activity of Hexahydro Acridine derivatives by DPPH Scavenging assay activity.

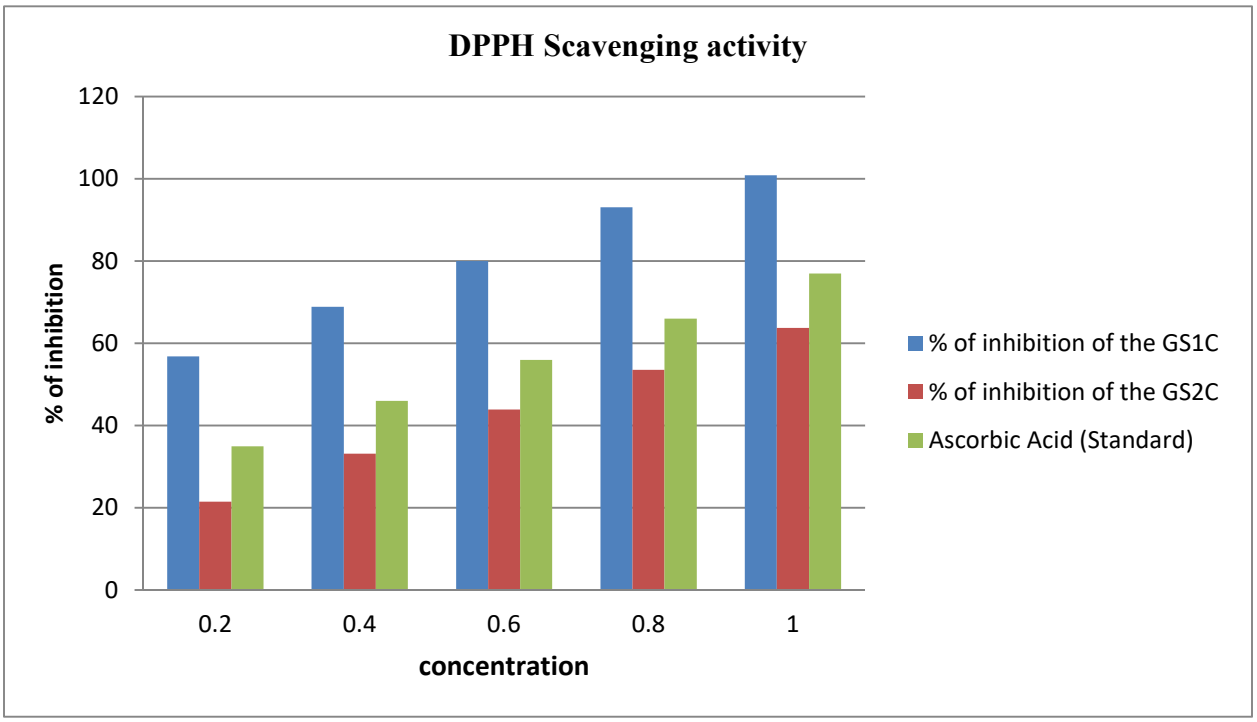


Fig.3 Graphical representation of Anti Oxidant activity of Hexahydro Aciridine derivatives by DPPH Scavenging assay activity

3.4 Anti- inflammatory activity

Inhibition of Albumen Denaturation method

There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available. Hence, in the present study, the protein denaturation bioassay was selected for in vitro assessment of the anti-inflammatory property of the derivatives of hexahydroacridine. The Albumen Denaturation is a well-documented cause of inflammation. Most biological proteins lose their biological functions when denatured. Production of autoantigen in certain arthritic disease is due to denaturation of protein. The mechanism of

denaturation involves an alteration in electrostatic hydrogen, hydrophobic, and disulfide bonding. In the presence study, denaturation of proteins is the main cause of inflammation. As part of the investigation on the mechanism of the anti-inflammatory activity, ability of the acridine to inhibit protein denaturation was studied. Selected acridine were effective in inhibiting heat-induced albumin denaturation. Aspirin was used as a standard antiinflammation drug as shown in Figure [Table 2 and Figure 4]. The albumin denaturation method was carried out at different concentrations of the derivatives of hexahydroacridine. samples, albumin denaturation, 100µg/ml 200µg/ml 300µg/ml 400µg/ml and 500 µg/ml.

For the 9-(4-Bromophenyl)-3,3,6,6 tetramethyl-3,4,6Tetramethyl 3,4,6,7,9,10 – hexahydro– 2H, 5H –acridine 1,8 –Dione [GS1C], the result shows the percentage of cytotoxicity for 100 µg/ml as 37%, 200 µg/ml as 48%, 300 µg/ml as 60% , 400 µg/ml as 72% and 500 µg/ml as 82%. For the 9-(3-hydroxy 4-methoxy phenyl)-3,3,6,6 tetramethyl- 3,4,6,7,9,10-hexahydro-2H,5H- acridine- 1,8-dione [GS2C], the result shows the percentage of cytotoxicity for 100 µg/ml as 48%, 200 µg/ml as 61%, 300 µg/ml as 73.5% , 400 µg/ml as 86.5% and 500 µg/ml as 98.5%. These inhibition values are compared with standard drug of Aspirin for 100 µg/ml as 40%, 200 µg/ml as 50.5%, 300 µg/ml as 60% , 400 µg/ml as 70% and 500 µg/ml as 80.5%.

As a part of the investigation on the mechanism of the anti oxidant activity, ability of extract to inhibit Inhibition of Albumen Denaturation was studied. The in-vitro study of Antinflammatory activity indicates that the inhibition percentage of Albumen Denaturation by acrdine of 9-(3-hydroxy 4-methoxy phenyl)-3,3,6,6 tetramethyl- 3,4,6,7,9,10-hexahydro-2H,5H- acridine- 1,8-dione [GS2C] activity is higher than 9-(4-Bromophenyl)-3,3,6,6 tetramethyl-3,4,6Tetramethyl 3,4,6,7,9,10 – hexahydro– 2H, 5H –acridine 1,8 –Dione [GS1C].

S.No	Test	Concentration of the sample (µg/ml)	% of Protein Denaturation of the GS1C	% of Protein Denaturation of the GS2C	Aspirin (Standard)
1	Albumin denaturation	100	37	48	40
2		200	48	61	50.5
3		300	60	73.5	60
4		400	72.0	86.5	70
5		500	82.0	98.5	80.5

Table.2 Anti-inflammatory activity of Hexahydro Acridine derivatives by albumin denaturation

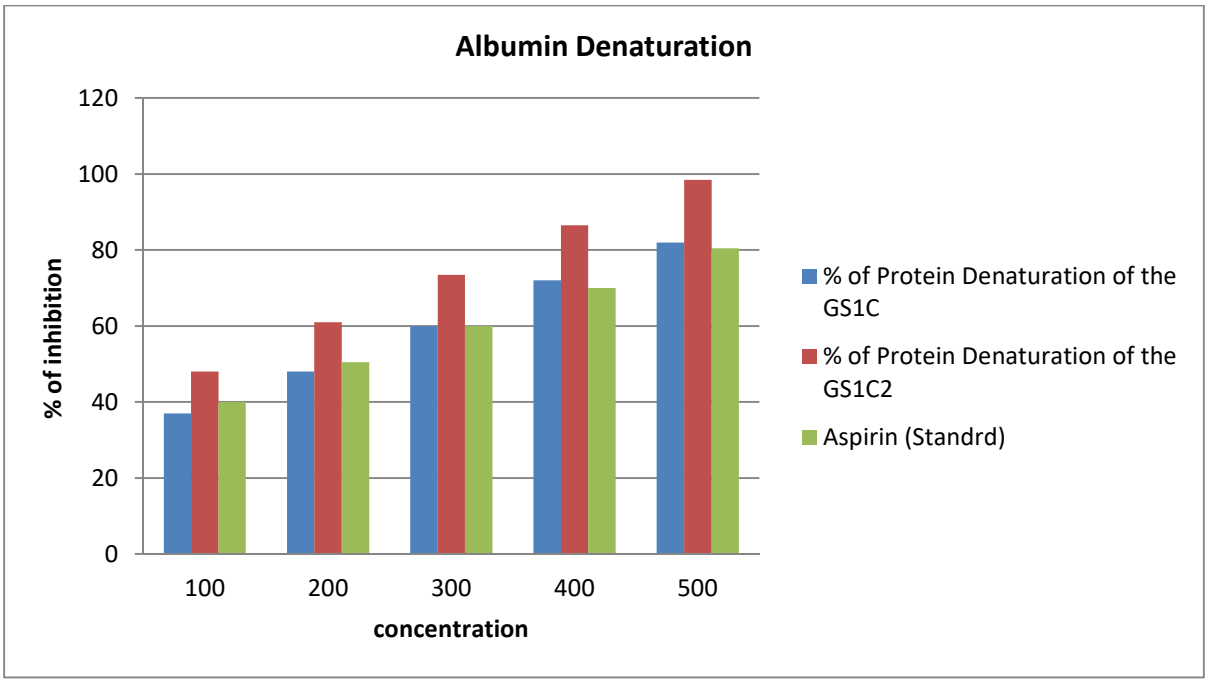


Fig.4 Graphical representation of Anti-inflammatory activity of Hexahydro Aciridine derivatives by albumin denaturation

3.5 Anti diabetic activity

Inhibition of Alpha-Amylase Enzyme

Diabetes mellitus is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. A therapeutic approach to decrease the hyperglycaemia is to inhibit the carbohydrate digesting enzymes (α-glucosidase and α-amylase), thereby preventing the breakdown of carbohydrates into monosaccharides which is a main cause of increasing blood glucose level. Therefore, developing compounds having inhibitory activities towards carbohydrate hydrolysing enzymes may be a useful way to manage diabetes. As shown in Figure 5 and Table 3, α-amylase and α-glucosidase were significantly inhibited in a dose-dependent manner by the 9-(4-Bromophenyl)-3,3,6,6 tetramethyl-3,4,6Tetramethyl 3,4,6,7,9,10 – hexahydro– 2H, 5H –acridine 1,8 –Dione [GS1C] and 9-(3-hydroxy 4-methoxy phenyl)-3,3,6,6 tetramethyl- 3,4,6,7,9,10-hexahydro-2H,5H- acridine- 1,8-dione [GS2C]. The results suggest that with the increased 9-(3-hydroxy 4-methoxy phenyl)-3,3,6,6 tetramethyl- 3,4,6,7,9,10-hexahydro-2H,5H- acridine- 1,8-dione [GS2C] and 9-(4-Bromophenyl)-3,3,6,6 tetramethyl-3,4,6Tetramethyl 3,4,6,7,9,10 – hexahydro– 2H, 5H –acridine 1,8 –Dione [GS1C], concentration, the activity levels of enzyme were remarkably reduced, Hence, the biomolecules likely enhanced the antidiabetic potential of the synthesized compounds. α-Amylase inhibitory actions were observed in increasing order, as Acarbose (Figure 5). Comparable results were observed. However, the foregoing results suggest that the synthesized hexahydroaciridine derivative are potentially better antidiabetic particles at inhibiting carbohydrate digesting enzymes, and could prove an effective approach in the diabetes care. For the 9-(4-Bromophenyl)-3,3,6,6 tetramethyl-3,4,6Tetramethyl 3,4,6,7,9,10 – hexahydro– 2H, 5H –acridine 1,8 –Dione [GS1C], the result shows the percentage of cytotoxicity for 20 µg/ml

as 15%, 40 µg/ml as 25.9%, 60 µg/ml as 39.5% , 80 µg/ml as 52.5% and 100 µg/ml as 64.2%. For the 9-(3-hydroxy 4-methoxy phenyl)-3,3,6,6 tetramethyl- 3,4,6,7,9,10-hexahydro-2H,5H-acridine- 1,8-dione [GS2C], the result shows the percentage of cytotoxicity for 20 µg/ml as 48%, 40 µg/ml as 61%, 60 µg/ml as 73.5% , 80 µg/ml as 86.5% and 100 µg/ml as 98.5%.

These inhibition values are compared with standard drug of Acarbose for 20 µg/ml as 40.2%, 40 µg/ml as 52.5%, 60 µg/ml as 64.8% , 80 µg/ml as 76.9% and 100 µg/ml as 89.7%. The Alpha-Amylase Enzyme was carried out at different concentrations of derivatives of hexahydroacridine derivatives namely Alpha-Amylase Enzyme 20 µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100 µg/ml. Albumen Denaturation did not show any significant difference at 20 µg/ml and 40µg/ml,Schiff base, however, it was significant for 0.15µg/ml, 0.20µg/ml and 0.25 µg/ml for the nanoparticles, all the values are compared with standard drug of Acarbose (Figure 5). Antidiabetic activity of synthesized GS2C based on inhibition of αamylase and activity.

As a part of the investigation on the mechanism of the Anti diabetic activity, ability of hexahydroaciridine to Inhibition of Alpha-Amylase Enzyme was studied. The in-vitro study of Anti diabetic activity indicates that the inhibition percentage of Alpha-Amylase Enzyme by activity is 9-(3-hydroxy 4-methoxy phenyl)-3,3,6,6 tetramethyl- 3,4,6,7,9,10-hexahydro-2H,5H-acridine- 1,8-dione higher .

S.No	Test	Concentration of the sample (µg/ml)	% of inhibition of the GS1C	% of inhibition of the GS2C	Acarbose
1	Alpha amylase inhibitory activity	20	15.0	48	40.2
2		40	25.9	61	52.5
3		60	39.5	73.5	64.8
4		80	52.5	86.5	76.9
5		100	64.2	98.5	89.7

Table.3 Anti diabetic activity of Hexahydro Acridine derivatives by Alpha amylase inhibitory activity

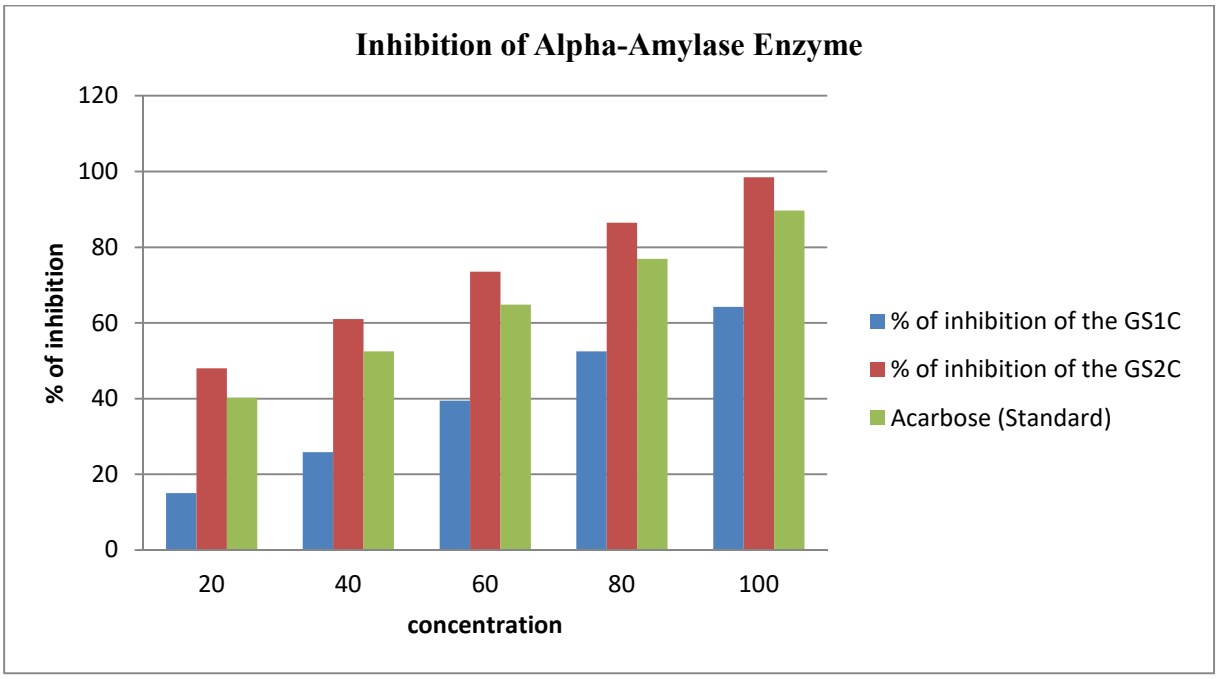


Fig.5 Graphical representation of Anti diabetic activity of Hexahydro Acridine derivatives by Alpha amylase inhibitory activity

4.CONCLUSION

The illustrated synthetic protocol allow for the research of a cycle of hexahydroacridine derivatives as illustrated an outstanding optical properties.

The condensation of 5,5-dimethylcyclohexane-1,3-dione and ammonium acetate different substituted aromatic aldehyde 3-hydroxy 4-methoxybenzaldehyde, 4-Bromo benzaldehyde. The Componud is synthesized by simple condensation in ethanol. Hexahydroaciridine were synthesized and characterized. The synthesized compounds studied for their *in vitro* antioxidant, anti-inflammatory and anti-diabetic activity. The DPPH assay is the most acceptable, fastest and simplest method for the calculation of the free radical scavenging activity. As shown in the Table 1 and Figure 3. The GS2C shows better antioxidant property than the standard ascorbic acid with an IC50 values. The results from DPPH method revealed that compounds are capable of donating electron or hydrogen atom and subsequently react with free radicals or terminate chain reactions in a dose-dependent pattern.

Denaturation of proteins is a well-documented cause of inflammation. Phenylbutazones, salicylic acid, flufenamic acid (anti-inflammatorydrugs), have shown dose dependent ability to thermally induced protein denaturation. As a part of the investigation on the mechanism of the anti-inflammatory activity, ability of extract to inhibit protein denaturation was studied. The invitro study of a anti-inflammatory activity indicates that the inhibition percentage of derivatives of hexahydroacridine α-amylase is a key enzyme in carbohydrate metabolism. Inhibition of α-amylase is one of the strategies for treating diabetes. Amylase inhibitors are also known as starch

blockers because they contain substances that prevent dietary starches from being absorbed by the body. The anti-diabetic study of these compounds may reduces the postprandial glucose level in blood by the inhibition of alpha-amylase enzymes, which can be an important strategy in management of blood glucose. Based on the result, it is clearthat these compounds can be used as antioxidants, antidiabetic drug in the field of medicinal and food industry.

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