In-vitro Antidiabetic activity of synthesized 1,2,3,4-tetrahydrocarbazole and its derivatives

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Abstract: Diabetes mellitus is a metabolic disorder causing illness throughout the world. Insulin plays a vital role in control of glucose homeostasis. Deficiency of insulin in body affects nutrients metabolism. Treatment of diabetes with less or no side effects is still a challenge to the medical scientist. Thus, the present communication attempts to evaluate in-vitro antidiabetic potential of various previously synthesized 1,2,3,4-tetrahydrocarbazole moiety along with their different derivatives. The methods include treatment of various concentrations of synthesized compounds with enzymes alpha-amylase and alpha-glucosidase in order to check the percent inhibition. As it is known that inhibition of the activity of such alpha-amylase and alpha-glucosidase would delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose, as a result the reduction of postprandial blood glucose level elevation. The assay results suggested that the compound(s) exhibit dose dependent increase in inhibitory effect on both enzymes. Therefore study reveals that these synthesized compound(s) could be an important strategy in management of blood glucose.

INTRODUCTION
Carbazole having a wide range of applications in various fields such as biological, synthetic and industrial (Kaushik K et al., 2012). It has having many medicinal properties like antioxidant (Haider N et al., 2001) antimicrobial (Jain V et al., 2012), antidiabetic (Vairappan CS et al., 2011), antihyperlipidemic, antiinflammatory, anticancer (Nahari et al.,2013, Mohammad A et al., 2001, Sangeetha V et al.,2013). Other than this, carbazole derivatives also having application as organic semiconductor materials for the fabrication of organic field-effect transistors (OFET) and polymeric-optoelectronic materials (Park M et al., 1998). Wide range of activities of carbazole nucleus has encouraged the researchers to explore for synthesis of novel derivatives having the possible biological activity. Diabetes mellitus is a composite and a diverse group of disorders that disturbs the metabolism of carbohydrate and other body nutrients. According to the latest report number of diabetes mellitus cases has been increasing worldwide. In 2000, WHO estimated a total of 171 million of people with diabetes mellitus from the global population, and this report projected to increase to 366 million by 2030 (Wild S et al., 2004). The treatment of diabetes need to spent vast amount of resources including medicines, diets, physical training and along with serious complications often resulting in high death rate. Therefore there is a need for searching of a new class of compounds to overcome diabetic problems (Syamsudin S, 2010). Thus taken above into considerations previously synthesized compounds were screened for their in-vitro antidiabetic activity and to find out the comparative potential of the compounds.

MATERIAL METHODS
All the chemicals were procured by Himalayan Institute of Pharmacy and Research, Dehradun (Manufacturer Central Drug House, New Delhi). The entire synthesized compound was checked for Thin layer chromatography (TLC) on pre-coated TLC plates (Merck) and spots were detected through exposure to (Ultra-Violet) UV-lamp chamber at 254nm, 365nm and visible light. Finally post-derivertised by freshly prepare KMnO4 solution. The 1H-NMR spectra were recorded by using Dimethyl sulfoxide (DMSO) as a solvent.

Table no- 1: Synthesized compound 1

<table>
<thead>
<tr>
<th>Comp. (1)</th>
<th>Comp.name</th>
<th>Structure</th>
<th>wt.</th>
<th>Mol.wt</th>
<th>Eq</th>
<th>Mole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cyclohexone</td>
<td><img src="image" alt="Cyclohexone" /></td>
<td>8.8g, 11.31ml</td>
<td>84.16gm/mol</td>
<td>1</td>
<td>0.104</td>
</tr>
<tr>
<td></td>
<td>Phenylhydrazone</td>
<td><img src="image" alt="Phenylhydrazone" /></td>
<td>11.24g, 10.25ml</td>
<td>108.14gm/mol</td>
<td>1</td>
<td>0.0804</td>
</tr>
</tbody>
</table>
In vitro methods employed in antidiabetic studies

**Inhibition of alpha-amylase enzyme**

A starch solution (0.1% w/v) was obtained by stirring 0.1g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha-amylase 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. Both control (Acarbose std. drug) and synthesized compound(s) were added with starch solution and left to react with alpha- amylase solution under alkaline conditions at 25°C. The reaction was measured over 3 minutes. 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 CP & Singh MB, 1980).
Inhibition of alpha-glucosidase enzyme
The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 1 ml with 0.2 M Tris buffer pH 8.0 and various concentration of control (Acarbose std. drug) and the synthesized compound(s) for 5 min at 37°C. The reaction was initiated by adding 1 ml of alpha-glucosidase enzyme (1U/ml) to it followed by incubation for 40 min at 35°C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. Then the intensity of the colour was measured at 540nm (Krishnaveni S, 1984).

Calculation of 50% Inhibitory Concentration (IC50)
The concentration of the synthesized compounds required to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by I % = (Ac-As)/Ac X 100 (Shai LJ, 2010). Here Ac = absorbance of the control and As = absorbance of the sample.

RESULTS AND DISCUSSIONS
All the synthesized compound(s) were tested for their in vitro antidiabetic potential at different concentrations from 0.2 to 1.0 ml by using enzymes alpha-amylase and alpha-glucosidase in order to check their percent inhibition. All compound(s) shows dose dependent increase in percentage inhibition. Results are shown in Table no-6 and Table no-7.

CONCLUSION
The present study attempts to investigate in-vitro antidiabetic activity of synthesized 1,2,3,4-tetrahydrocarbazole and its derivatives. Total six compounds 1,1a, 1a’, 1c, 1c’ and 1c” were previously synthesized and tested for their in vitro antidiabetic potential. The present findings divulge that all synthesized compound(s) efficiently inhibits both alpha amylase and alpha glucosidase enzymes in vitro in a dose dependent manner. Data reveals all compounds have significant inhibitory activity, whereas compound 1c’ found to be most active against both enzymes. Further studies are required to validate the data through in-vivo antidiabetic activity.

REFERENCES
Mohammad A., Khan AT., Khan KM., Islamia MJ.,


