Research Article

Dual inhibition of cholinesterase enzyme by an aqueous extract of *Hibiscus rosa sinensis* L.

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ABSTRACT

Among many Indian medicinal plants, *Hibiscus rosa sinensis* has been used from centuries in treating liver disorder, high blood pressure, headache and other diseases associated with central nervous system. In Indian ayurvedic system of medicine this plant has been considered to be a brain tonic. In present study, an aqueous extract of *Hibiscus rosa sinensis* was assessed for their anti-cholinesterase activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BUChE). Ellman's assay was used for inhibition study of cholinesterase enzyme. Our study demonstrated that an aqueous extract of this plant significantly inhibited both AChE and BUChE enzymes in a concentration dependent manner. An aqueous extract of *Hibiscus rosa sinensis* showed 62.02%±0.03 (SEM) inhibitory activity against AChE and 57.83%±0.05 (SEM) inhibitory activity against BUChE enzymes respectively. The IC50 value calculated from the percentage inhibition curve was 48.61μg/mL and 70.52μg/mL against AChE and BUChE respectively. These result demonstrated that an aqueous extract of *Hibiscus rosa sinensis* moderate AChE and BUChE activity. In conclusion, the present study indicated that an aqueous extract of *Hibiscus rosa sinensis* has got a potential dual anti-cholinesterase activity and can be explored further for isolation of potential therapeutic phytoconstituents that will be useful in improving memory and other cognitive function associated with the cholinergic system.

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INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease of the brain which affects more than 37 million people all over the world [1]. The symptoms associated with AD are memory loss, language deterioration, akinesia, mutism, ideational and ideomotor apraxias found in elderly people [2]. A deficit of the brain acetylcholine (ACh) affects the normal functioning of the brain cholinergic system. Downregulation of AChE as well as BUChE activity can overcome the decrease level of ACh. AChE and BUChE inhibitors block the degradation of ACh by inhibiting the activity of AChE enzyme which subsequent result into the increase in the cholinergic transmission [3]. Blocking of AChE activity is considered to be a suitable approach for symptomatic treatment of AD [4].

Hibiscus rosa sinensis L. (Malvaceae) is an evergreen, herbaceous plant also known as shoe flower. It is an ornamental plant, native to the tropical and subtropical region of the world [5]. A number of scientific studies demonstrated that *Hibiscus rosa sinensis* plant possess significant free radical scavenging activity [6]. The flower and leaves of the plant were found to exhibit significant hypoglycemic [7], lipid lowering [8], and cardioprotective activity [9]. Moreover the plant also reported to have an anti-spermatogenic [10], anti-tumor [11] and anti-convulsant [12] activities. The different studies reported number of chemical constituent in a *Hibiscus rosa*

sinensis plant such as anthocyanin, anthocyanidine, quarcetin, carotene, niacin, riboflavin, lauric acid, gentisic acid, margaric acid, malvalic acid [13]. At present only few synthetic drugs such as donepzil, rivastigimine and other natural product based galanthamine available for treatment of memory loss and cognitive dysfunction associated with AD. These synthetic agents are often associated with common side effect in AD patients [14]. Due to increase incidence of side effects of synthetic medicine, more research will be focused toward the natural resources.

MATERIALS AND METHODS

Chemicals and Reagents

Acetylthiocholineiodide (ATChI), acetylcholineesterase (AChE) (EC 3.1.1.7), butyrylthiocholine iodide (BTChI), butyrylcholinesterase (BUChE) (EC 3.1.1. 8), 5, 5- dithiobis [2-nitrobenzoic acid] (DTNB), sodium bicarbonate (Sigma, Himedia) and phosphate buffer.

Plant Materials

The flower samples (**Fig. 1**) of plant *Hibiscus rosa sinensis* (Voucher no. MN/USBT51) were collected from natural sources. The specimens of this plant sample are stored in a herbarium at USBT, GGSIP University, Delhi, India.

Figure 1: Flower of *Hibiscus rosa sinensis*

Equipments and instruments

96-well plate (purchased from Corning Inc. NY), eppendorf tubes, centrifuge tubes, tips (Purchased from Tarsons products ltd. India), eppendorf tube stand, pipettes (purchased from Biomate), weighing balance, aluminium foil, tissue paper, ice box, blotting paper, vortex machine (CM101 purchased from REMI), magnetic stirrer (5MLH purchased from REMI) spatula, muslin cloth, spectrofluorometer (SpectraMax), centrifuge machine (Sigma) and lyophilizer (Thermo scientific).

Preparation of plant extract

The fresh sample of plant material was air dried at room temperature and powdered using electric grinder. 2 gm of sample was weighed and extracted with 40 ml of distilled water. The samples were filtered using muslin clothes, which was then freeze dried in lyophilizer. The sample was collected and kept in -20°C.

Cholinesterase inhibitory assay

AChE/BUChE inhibition was determined by the spectrophotometer using the Ellman's method with slight modification in other papers [15,16]. Cholinesterase inhibition was evaluated using the colorimetric method in flat-bottom 96- well microtitre plates. A typical procedure consisted of 5μl of AChE/BUChE solution, at final assay concentration of 0.08 U/ml; 5μl of the test extract; 200 μl of 0.1 M phosphate buffer pH 7 and 5μl of DTNB at a final concentration of 0.5mM prepared in 0.1 M phosphate buffer pH 7 containing 0.12 M of sodium bicarbonate. The final assay concentration used for an aqueous extract of the plant material was 100μg/ml. The reactants were mixed and pre-incubated for 15 min at 30°C. The reaction was initiated by adding 5μl of ATChI or BTChI at a final concentration of 0.5mM. As a control the inhibitor solution was replaced with buffer. The control was assayed in triplicate. For each run two blanks were prepared in triplicate to observe any non-enzymatic hydrolysis in the reaction mixture. One blank consisted of buffer replacing substrate and a second blank had buffer replacing enzyme. Change in absorbance of reactants mixture at 412 nm was measured on spectrophotometer, 96 well plate reader for a period of 2 min at 25°C. The reaction involved in this is enzyme which hydrolyses the substrate BTChI or ATChI resulting in the product thiocholine which reacts with Ellman's reagent (DTNB) to produce 2- nitrobenzoate-5-mercapto-thiocholine and 5-thio-2-nitrobenzoate which can be detected.

Quantitative measurement of IC$_{50}$ value by constructing a dose response curve:

Dose response curve was constructed by plotting the inhibition curve between different concentrations of *Hibiscus rosa sinensis* extract ranging from 6.125 to 100μg/mL (final assay concentration) and

percentage inhibition. Each concentration was assayed in triplicate (*n = 3)*. Microsoft EXCEL software was used to obtain the dose response curve. IC_{50} value calculated from the standard curve equation demonstrated the concentration of *Hibiscus rosa sinensis* extract required to inhibit the hydrolysis of substrate by 50%.

RESULTS

The results showed that an aqueous extract of *Hibiscus rosa sinensis* inhibited both AChE and BUChE in a concentration-dependent manner. The maximum inhibition 62.02%±0.05 (SEM) against AChE and 57.83%±0.03 (SEM) against BUChE was observed at the final assay concentration of 100μg/mL. The IC_{50} value calculated from the equation obtained from the log concentration versus inhibition curve was 48.61μg/mL against AChE and 70.52μg/mL against BUChE respectively (**Fig. 2 & Fig. 3**).
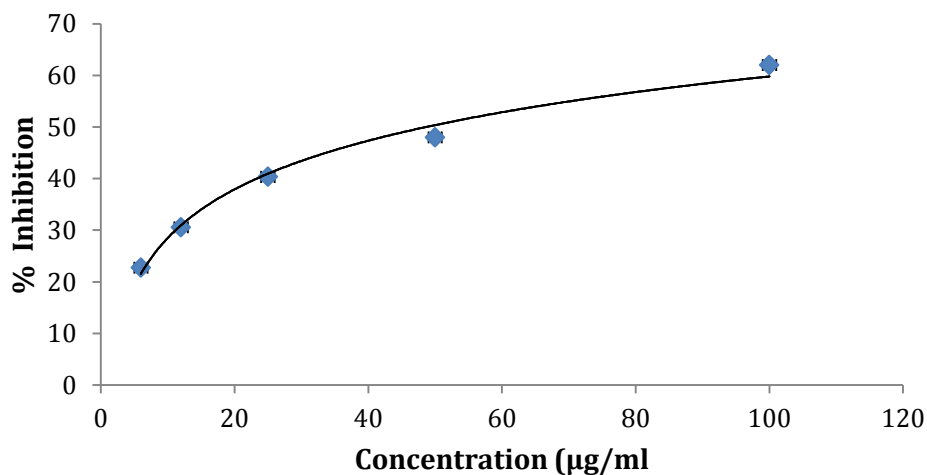

Figure 2: Concentration-dependent AChE inhibition by an aqueous extract of *hibiscus rosa sinensis*. (The equation for the line is $y = 13.60\ln(x) - 2.833$; $R^2 = 0.986$)
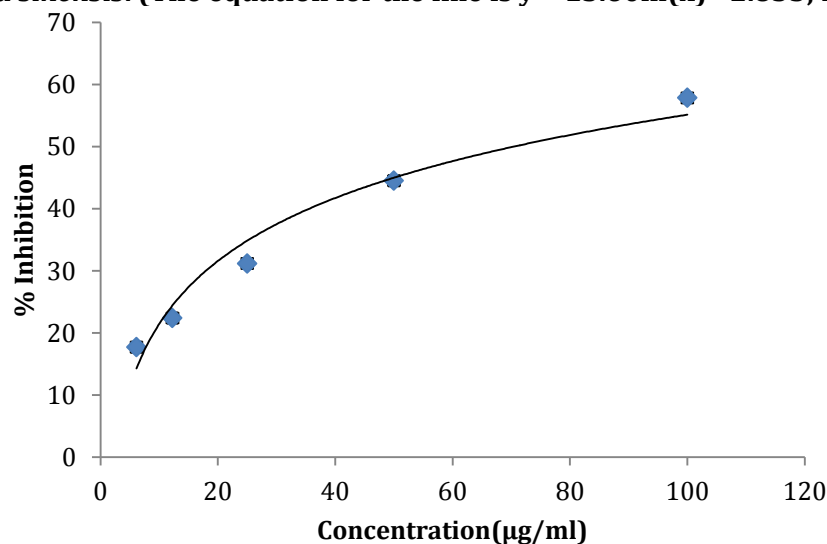

Figure 3: Concentration-dependent BUChE inhibition by an aqueous extract of *Hibiscus rosa sinensis*. (The equation for the line is $y = 14.63\ln(x) - 12.27$; $R^2 = 0.966$)

DISCUSSION

Various drugs categorized as cholinesterase inhibitors such tacrine, donepezil, rivastigmine and galantamine used for the symptomatic treatment of AD. The main drawbacks associated with these drugs are their adverse effects such diarrhea, nausea, muscles cramps, vomiting, fatigue, loss of appetite and hepatotoxicity. For these reason there is always a need for new safer molecules from natural resources which might have lesser side effects as compared to synthetic molecules. In the present study, an aqueous extract of *Hibiscus rosa sinensis*

showed significant dual inhibition of AChE and BUChE enzyme in a concentration dependent manner. This study is complementary to previous studies that demonstrated management of cognitive disorder and protective role in scopolamine induced amnesia [17]. Furthermore, another study showed protective role *Hibiscus rosa sinensis* against reserpine-induced orofacial dyskinesia and oxidative stress. This plant extract significantly reduced the lipid peroxidation and reversed the decrease in brain SOD, CAT and GSH levels [18]. *In vivo* neuroprotective effect of *Hibiscus rosa sinensis* in an oxidative stress model of cerebral post-ischemic reperfusion injury in rats which leads to deficits of learning and memory. The *Hibiscus rosa sinensis* extract ameliorated anxiety and there was improvement of learning and memory [19]. Our study complementary to previous neuronal activity in which dual anticholinesterase activity was demonstrated by *Hibiscus rosa sinensis* extract which might be benficla in ameliorating the symptoms associated with Alzheimer's disease.

CONCLUSION

In conclusion, an aqueous extract of *Hibiscus rosa sinensis* showed dual anticholinesterase activity. Further studies are required to identify, isolate and characterize the phytoconstituents from an aqueous extract to find novel molecule which might be useful in alleviating the symptoms associated with AD.

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