

Kinetics of butyrylcholinesterase inhibition by an ethanolic extract of *Shorea robusta*

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ABSTRACT

Purpose: Cholinesterase inhibitors are the class of compounds which inhibit cholinesterase enzyme. These are used as drugs for symptomatic treatment of Alzheimer's disease (AD). The present study, evaluate anti-butrylcholinestease property of ethanolic extract of *Shorea robusta* de oil cake (DOC).

Method: Ellman's method was used to determine the butyrylcholinesterase (BuChE) enzyme inhibitory activity by an ethanolic extracts of *Shorea robusta* de oil cake (DOC).

Results: In present study, *Shorea robusta* DOC showed concentration dependent BuChE inhibition by an ethanolic extracts of *Shorea robusta* with maximum inhibition ($63.11 \pm 0.09\%$) at $200 \mu\text{g/ml}$ final concentrations. The Lineweaver-Burk plot of an ethanolic extract of *Shorea robusta* DOC showed mixed non-competitive mode of inhibition.

Conclusion: The anti BuChE enzyme activity exhibited by an ethanolic extracts of *Shorea robusta* DOC extracts might be used in future for symptomatic treatment of AD.

Key words: Butyrylcholinesterase, *Shorea robusta*, Inhibition, Kinetics, Alzheimer's disease.

INTRODUCTION:

Alzheimer's disease (AD) is the most common form of dementia associated with symptoms such as confusion, irritability, aggression, mood swings, trouble with language, and long-term memory loss. AD is one of the several disorders that cause the gradual loss of brain cells affecting mostly elderly people [1], [2]. AD is characterized by disruption of synaptic function and massive loss of neurons throughout the brain, starting from the hippocampus area of the cortex that is important for the formation of memories [3]. Cholinesterase inhibitors are the drugs that prolong the existence of acetylcholine (ACh) after it is released from cholinergic nerve endings by inhibiting both acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzymes. BuChE, also known as plasma cholinesterase, pseudocholinesterase, acylcholineacylhydrolase found primarily in the liver. BuChE preferentially acts on butyrylcholine (BuCh), but also hydrolyzes ACh, BuChE is selectively inhibited by 10-[2-diethylaminopropyl]-phenothiazid (ethopropazine) and isotetramonoisopropyl pyrophosphate tetramid. BuChE hydrolyses BuCh into butyric acid and choline [4].

Cholinesterase inhibitors are used as drugs in the market for symptomatic treatment of AD since 1990. Cholinesterase inhibitors play efficacious role in treating the cognitive and functional symptoms associated with AD [5]. These are tacrine (an aminoacridine), donepezil (a benzylpiperidine), rivastigmine (a carbamate) and galantamine (a tertiary alkaloid). They appear similar in efficacy and their clinical differentiation may depend on differences in tolerability profiles and ease of use [5], [6]. The differences in the tolerability profiles of cholinesterase inhibitors may arise due to the differential selectivity for AChE and BuChE [7], [8]. The most common side effects of these drugs include diarrhoea, nausea, insomnia, muscles cramps, vomiting, fatigue and loss of appetite. In clinical studies these effects were often mild and generally went away with continued treatment [9]. There are some evidences that suggest BuChE activity may be involved in the pathogenesis of AD [10].

Sal (*Shorea robusta*) belongs to Dipterocarpacea family is a large sub-deciduous tree. It is native to southern Asia. In India it is densely populated in states likely Orissa, Madhya Pradesh, Bihar, Uttar Pradesh and West Bengal. Sal DOC is the by product of Sal oil industry and is generated after the Sal seed is crushed to squeeze the oil. Sal DOC contains approximately 5.4% of moisture, 94% of dry matter, 34% of crude protein, 1.7% of lipid, 1.9% of crude fibre, 4% of ash, 54% of total carbohydrates and 3.4% of tannic acid [11]. There is huge quantity of Sal seed product available which is not elsewhere utilized for cost effective purposes due to presence of tannins. This tannin containing residue must be detoxified by mixing with other substrate for further use, so they may have ability to hydrolyse the enzymes. Due to the fast growing interest in the medicinal plants by pharmaceutical companies and scientific research on the discovery of medicinal compounds, Sal could potentially play role in the production of new drugs of having medicinal properties with reduced side effects. A variety of chemical types are often present in the plant extract. They often have different biological or

pharmacological activities due to the presence of various tannins in *Shorea* sp. may be found good candidate as enzyme inhibitors for present study [12].

MATERIALS AND METHODS:

2.1 Chemicals and Reagents

Butyrylthiocholine iodide (BTChI), butyrylcholinesterase (BuChE) (EC 3.1.1.8), 5, 5- dithiobis [2-nitrobenzoic acid] (DTNB), sodium bicarbonate (Sigma, Himedia), phosphate buffer, ethanol (SRL)

2.4 Preparation of plant extract

The plant material were collected from the local market and identified by the local botanist and voucher specimen number CK/USBT002 stored in the lab. The plant material was air- dried at room temperature and powdered using electric grinder. 1 gm of sample was weighed and extracted with 25 ml of ethanol (70%). The samples were filtered using muslin clothes, which was then concentrated by the help of rotary evaporator. The samples were collected and kept in -20°C.

2.5 Cholinesterase inhibitory assay

BuChE inhibition was determined by the spectrophotometer using the Ellman's method with slight modification in other papers [13], [14]. An assessment of cholinesterase inhibition was carried out in flat-bottom 96- well microtitre plates using the colorimetric method. A typical run consisted of 5µl of BuChE solution, at final assay concentration of 0.08 U/ml; 200 µl of 0.1 M phosphate buffer pH 8; 5 µl 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; and 5 µl of the test extract. The final assay concentration used for an ethanolic extract of the plant material was 200 µg/ml. The reactants were mixed and pre-incubated for 15 min at 30°C. The reaction was initiated by adding 5 µl 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. To monitor any non- enzymatic hydrolysis in the reaction mixture two blanks for each run were prepared in triplicate. One blank consisted of buffer replacing enzyme and a second blank had buffer replacing substrate. Change in absorbance at 415 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 hydrolyses the substrate BuCh resulting in the product thiocholine which reacts with Ellman's reagent (DTNB) to produce 2- nitrobenzoate-5- mercaptothiocholine and 5-thio-2-nitrobenzoate which can be detected.

RESULTS:

The results showed that an ethanolic extract of *Shorea robusta* at concentration ranging from 12.5 to 200µg/mL showed concentration-dependent inhibition of enzyme BuChE. The maximum inhibition of 63.11±0.09% for enzyme BuChE was observed at 200µg/mL final assay concentration. The IC₅₀ value calculated from the equation obtained from the concentration versus percentage inhibition curve was 70µg/mL (Figure 1).

The mode of enzyme inhibition was derived from the Lineweaver-Burk (LB) plot between the reciprocal of substrate concentration on x-axis and reciprocal of velocity on y-axis [15]. The LB plot of an ethanolic extract of *Shorea robusta* DOC showed mixed non competitive inhibition kinetics as the intersection of lines occurred neither on x-axis or y-axis as illustrated in Figure 2.

5. DISCUSSION AND CONCLUSION

Cholinesterase inhibitors are currently used for symptomatic treatment of AD. Several synthetic cholinesterase inhibitors such as tacrine, rivastigmine, and donepezil are available in the market for symptomatic treatment and management of conditions related AD. Moreover, their side effects have become increasingly noticeable [16]. Due to this reason there is an urgent need for safer, more tolerable, more bioavailable and non-toxic anti-cholinesterase drugs derived from the natural products.

Shorea robusta L. (Dipterocarpaceae), popularly known as Sal or Shal, is widely used in Ayurveda and Unani medicine. A recent study with methanol extract of mature leaves reported anti-inflammatory and antinociceptive activity [17]. In the present study, an ethanolic extract of *Shorea robusta* showed significant inhibition of BuChE enzyme in a concentration dependent manner. The mechanism of inhibition derived from LB plot showed mixed non competitive inhibition kinetics.

In conclusion, the present study an ethanolic extract of *Shorea robusta* showed anti butyrylcholinesterase activity. Further studies are required to purify, isolate and characterize the ethanolic extract to find newer phytoconstituents which might be useful in alleviating the symptoms associated with AD.

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Figures

Fig 1: Percentage inhibition of BuChE activity at different concentration of an ethanolic extract of *Shorea robusta* (DOC). The equation of the line is $y=10.46 \ln(x) + 5.487$; $R^2=0.977$

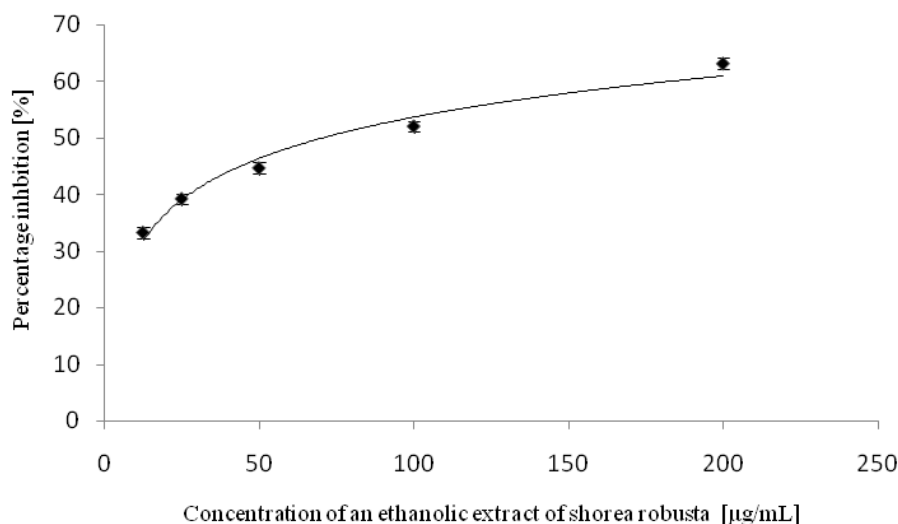


Fig 2: Lineweaver-Burk plot representing the reciprocal of initial enzyme velocity versus the reciprocal of butyrylthiocholine iodide concentration in the presence and absence (control) of different concentrations of an ethanolic extract of *Shorea robusta* (De oil cake).

