International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Anti-Acetylcholinesterase Activity of Methanolextract of *Calotropis Procera* Leaves

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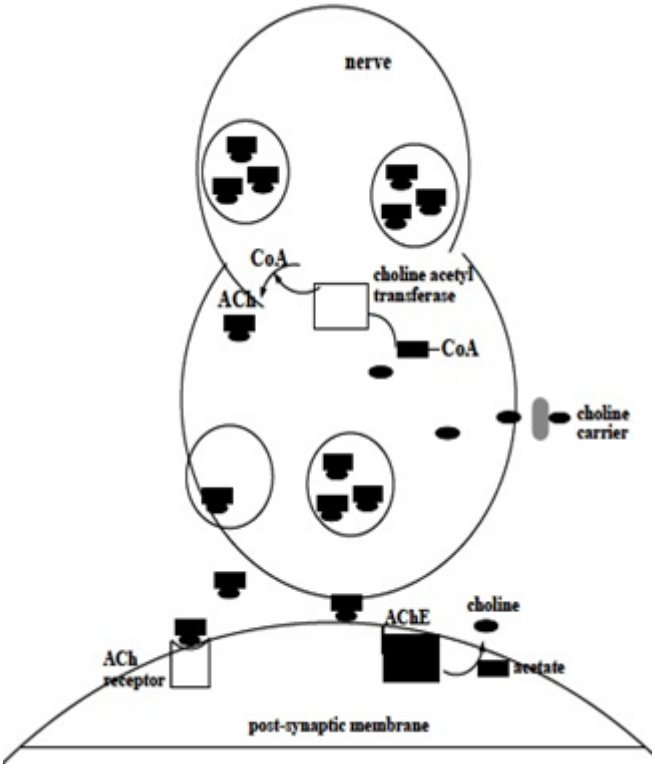
Abstract: ***Acetylcholinesterase (AChE), a serine hydrolase, catalyzes the breakdown of the neurotransmitter acetylcholine (Ach) into acetate and choline. This process involves the formation of substrate- enzyme complex, followed by acetylation of hydroxyl group of the amino acid serine, present within the esteratic site which is finally deacetylated. Invitro characterization of toxins and drugs including potential treatments of neurodegenerative diseasescan be determined using assay of acetylcholinesterase. The acetylcholinesterase activity, inhibitory activities and Lipid perioxidation of methanol extract of Calotropisprocera leaves were determined using ex-vivo standard methods. The methanol extract of Calotropisprocera leaves was analysed for acetylcholinesterase enzyme and the lipid perioxidation in the brain and liver. The acetylcholinesterase activity in the brain was higher than that of the catechin (Standard). The lipid perioxidation in the brain and liver was also higher than that of the standard.The methanol extracts of Calotropisprocera leaves may be used in the treatment of neurodegenerative diseases and Alzheimer diseases. The plantmay also be used as a cholinergic neurotransmitter.***

Keywords: *Calotropisprocera* leaves, Acetylcholinesterase, Alzheimer's disease, Lipid perioxidation

1. Introduction

One of the most important roles of acetylcholine in the brain is to govern the connectivity among neurons, thereby regulating the brain's cognitive functions. A deficit in acetylcholine, especially in the basal forebrains, is a neurochemical characteristic of patients clinically diagnosed with Alzheimer's disease (AD). Using acetylcholinesterase (AChE) inhibitors to retard the catabolic hydrolysis of acetylcholine, therefore compensating such deficiency particularly at the synaptic terminals, has been suggested as one of the most direct remedies for AD treatments. To date, only three AChE inhibitors; donepezil, rivastigmine, and galantamine, have been approved by US FDA for the treatment of AD.Whereas, it is arguable whether such drugs provide a long –term treatments or are merely a symptom intervention, they are one of only few effective approaches available for treatment of AD (Racchi, 2004).



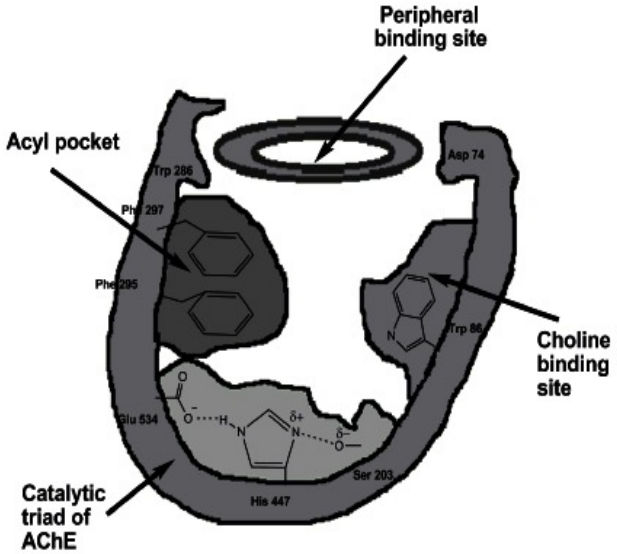


Figure 1: Schematic Representation of Ache Binding Site
Source: Tougu V,2001.

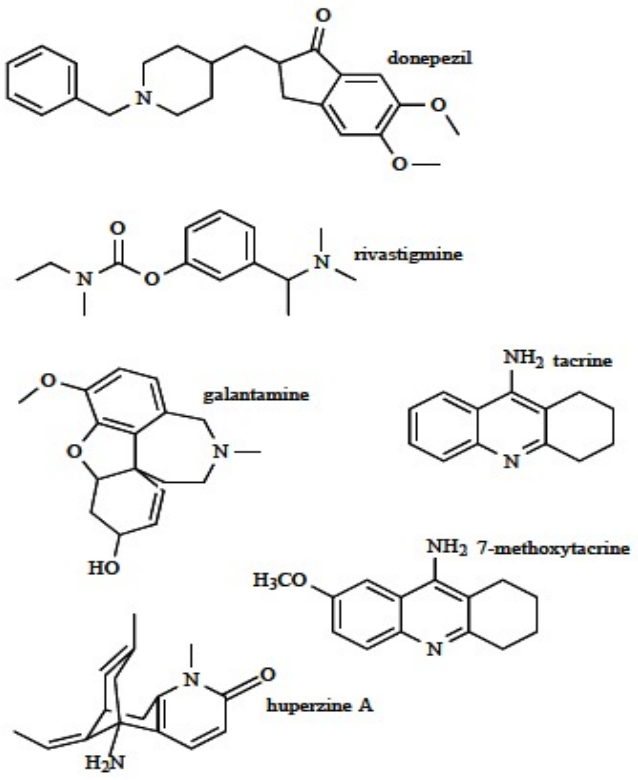


Figure 2 and Figure 3: Mechanism of Ache Action In Neurotransmission and Selected Reversible Ache Inhibitors in Pharmacotherapy of Ad; Source:Arce*et Al.*, 2009 And Castro *Et Al.,* 2006

Species of Calotropis are commonly known as 'The swallow-wort' or 'Milkweed' and belong to the family Asclepiadeace. Calotropis is regarded as useful medicinal plant and used in folk medicine. Although the latex of *Calotropisprocera* has been extensively studied and found responsible for cytotoxic, procoagulant, anti-inflammatory, abortifacient activities (Mhaskar*et al*., 2000). This plant is soft-wooded, evergreen, perennial shrub. It has one or few stems, few branches, and relatively few leaves, mostly concentrated near the growing tip. The bark is corky, furrowed, and light gray. A copious white sap flows whenever stems or leaves are cut. Giant milkweed has a very deep, stout taproot with few or no near-surface lateral roots. They were found to have few branches and reach depths of 1.7 to 3.0m in Indian sandy desert soils (Sharma, 1968).
The opposite sides of the leaves are oblong-obovate to nearly orbicular, short-pointed to blunt at the apex and have very short petioles below a nearly clasping, heart-shaped base. The leaf blades are light to dark green with nearly white veins. They are 7 to18cm long and 5 to 13cm broad, slightly leathery and have fine coat of soft hairs that rub off. The flower clusters are umbelliform cymes that grow at or near the ends of twigs. Extract of *CalotropisProcera* Leaves have shown great promise as nematicides, invitro and invivo (Anver and Alam, 1992). While several species of the genus Calotropis have been investigated, there is a dearth of information on the acteylcholinesterase activity and lipid perioxidation. Hence, the acetylcholinesterase activity and lipid prioxidation of the methanol extract of *Calotropisprocera* was investigated.

2. Material and Methods

2.1Plant Collection

Leaves of the *Calotropisprocera*was harvested from IkareAkoko, Ondo State in Nigeria, during the beginning of rainy season. They were authenticated at the University of Ibadan Botany Department. The plant materials were then washed separately with fresh water to remove dirty materials and was air dried for several days. The dried materials were ground into powder by grinding machine and the materials were stored at room temperature for future use.

2.2 Extract Preparation

About 500gm of powdered plant materials was taken and soaked with 2.5litres of methanol for 96 hours. The final extract was concentrated with a rotatory evaporator.

2.3 Chemicals

Distilled deionised water, DTNB (5'5'- Dithio-bis(2-nitrobenzoic acid), Acetylcholine iodide, methanol, HCL, Ascorbic acid, Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), di-sodium hydrogen phosphate $12H_20$, Tris Buffer, Sodium di-hydrogen phosphate $2H_20$ Disodium hydrogen phosphate, iron sulphate were purchased from sigma chemical USA.

2.4 Determination of Lipid Peroxidation Inhibition Assay

The lipid peroxidation inhibition assay (LPI) was determined according to the method described by (Liu *et al*., 2003) with a slight modification. Excised rat liver was homogenized in buffer and then centrifuged to obtain liposome. 0.5 ml of supernatant, 100 µl 10 mM FeSO4, 100 µl 0.1 mM Ascorbic Acid and 0.3 ml of extractives or standard at different concentration were mixed to make the final Volume 1 ml. The reaction mixture was incubated at 37°C for 20 minutes. 1 ml of (28%) TCA and 1.5 ml of (1%) TBA was added immediately after heating. Finally, the reaction mixture was again heated at 100°C for 15 minutes and cool at RT. After cooling, the absorbance was taken at 532 nm. Percentage inhibition of lipid peroxidation (% LPI) was calculated by the following equation,

$$\% I = [(A_{control} - A_{sample})/ A_{control}] \times 100$$

Where Acontrol is the absorbance of the control, and Asample is the absorbance of the extractives/standard. Then % of inhibition was plotted against concentration.

2.5 Determination of Acetylcholinesterase Inhibitory Activity

Aliquots of homogenates of individual rat brain of the various treatment groups were taken and used to measure acetyl cholinesterase activity, a marker for cholinergic neurotransmission (Ellman *et al*., 1961). Briefly, the acetylcholinesterase activity in the homogenate was measured by adding 2.6 ml of phosphate buffer (0.1M, pH 7.4), 0.1ml of 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) and 0.4ml of the homogenate. Then 0.1ml of

acetylthiocholine iodide solution was added to the reaction mixture. The absorbance was read using a spectrophotometer at wavelength of 412nm and change in absorbance for ten minutes at two minutes interval was recorded. The rate of acetylcholinesterase activity was measured by following the increase of colour produced from thiocholine when it reacts with DTNB. The change in absorbance per minute was determined and the rate of acetylcholinesterase activity was calculated and expressed as µmoles/min/g tissue.

$$\text{Rate}=\Delta\text{Abs/min}=\frac{A+B+C+D+E}{10}$$

Where A, B, C, D & E are Δabsorbance from 2mins to 10mins.
Enzyme Activity =%Rate
Percentage Inhibition = 100 -% enzyme activity

3. Results and Discussion

3.1 Lipid Perioxidation of *Calotropisprocera*

The lipid peroxides scavenging activities of C*alotropisprocera*leaves in rat liver homogenates was given in Tables 1. At concentration of 250- 1000 µg/ml, the scavenging activities of C*alotropisprocera* in rat liver was 43.3%, 82.1%, 83.6%, 91.2%; while that of Catechin(standard) was, 61,8%, 31.9% , 37.2% and 27.3%respectively. The lipid peroxides scavenging activities of C*alotropisprocera* leaves in rat liver was statistically higher than Catechin

The lipid peroxides scavenging activities of C*alotropisprocera* leaves in rat brain homogenates was given in Tables 2. At concentration of 750- 1000 µg/ml, the scavenging activities of C*alotropisprocera* in rat brain was , 26.1%, 34.1%, and 43.1%; while that of Catechin the standard was, 19.4%, 20.1% and 13.9% respectively. The lipid peroxides scavenging activities of C*alotropisprocera* leaves in rat brain was statistically higher than Catechin.

3.2. Acetylcholinesterase activity of Calotropis procera leaves

The acetylcholinesterase activity in rat brain of the methanolic extract of C*alotropisprocera* was compared with catechin (standard). The methanolic extracts of CAL had higher activity than that of catechin. At the concentration of 10-750ug/ml, the activity of CAL reached 76%, 74%, 73%, 70%, 78%, 71% while at the same concentration, that of catechin was 1.7%, 30%, 37.5%, 11.3%, 15%, 31%. This shows that CAL had a better activity effect than the standard (Catechin)

Table 1: The Scavenging Activities of C*alotropisprocera*leaves Extracts On Iron/Ascorbate-Induced Lipid Perioxidation (Lpo) In Rat Liver Homogenate *In Vitro*

Percentage Inhibition of LPO (%)

CONC(UG/ML)	CATECHIN	CPL
Control	0.00±0.00	0.00±0.00
10	34.04±1.36*	76.73±9.81*
50	47.61±1.81*	17.24±80.96*
100	50.23±2.95*	14.40±104.87*
250	61.81±7.57*	43.27±64.69*
500	31.85±8.73*	82.07±6.50*
750	37.17±4.91*	83.64±6.90*
1000	27.29±1.92*	91.22±3.10*

Data are expressed as mean ± SD (n=4)
* Significantly different from control ($p<0.05$)
CPL; *Calotropisprocera* leaves

Lipid peroxidation (LPO) involves the formation and propagation of lipid radicals with numerous deleterious effects, such as destruction of membrane lipids, metabolic disorders and inflammation. Production of malondialdehyde (MDA) is the end product of LPO. This process is initiated by hydroxyl and superoxide radicals leading to the formation of peroxy radicals that ultimately propagates chain reaction in lipids. Thus, antioxidants which are capable of scavenging hydroxyl, superoxide or peroxy radicals could prevent LPO. Results from this study, showed that extracts from *Calotropisprocera* inhibits LPO in rat liver and brain homogenates. *Calotropisprocera* protected against LPO induced by Fe^{2+}, considerably reduced MDA content in a concentration-dependent manner in rat liver and brain. CPL had the greatest inhibiting activity (91.2% in rat liver homogenate and 43.1% in rat brain homogenate) when compared to the activity of standard, (Catechin; 27.3% in rat liver homogenate and 20.1% in rat brain homogenate).

Table 2: The Scavenging Activities of C*alotropisprocera* Leaves Extracts On Iron/Ascorbate- Induced Lipid Perioxidation (Lpo) In Rat Brain Homogenate *In Vitro*

Percentage Inhibition of LPO (%)

CONC(UG/ML)	CATECHIN	CPL
Control	0.00±0.00	0.00±0.00
10	6.57±5.11*	29.45±7.29*
50	16.50±19.46*	10.80±9.17*
100	28.41±0.23*	18.65±8.63*
250	5.40±70.50*	0.08±20.42
500	19.42±18.59*	26.11±5.87*
750	20.05±19.48*	34.19±10.58*
1000	13.85±12.80*	43.36±20.62*

Data are expressed as mean ± SD (n=4) ,* Significantly different from control ($p<0.05$) , CPL; *Calotropisprocera* leaves

Table 3: Determination of Acetylcholinesterase Activity in Rat Brain Acetylcholinesterase Activity

Conc(ug/ml)	*Catechin*	*CPL*
Control	8.75	8.75
10	1.67	76*
50	30*	74*
100	37.5*	73*
250	11.25*	70*
500	15*	78*
750	31*	71*
1000	96*	70*

Data are expressed as mean ± SD (n=4),* Significantly different from control ($p<0.05$) CPL; *Calotropisprocera* leaves. Acetylcholine is invoved in the signal transfer in the synapses.After been delivered in the synapses, acetylcholine is hydroylsed giving choline and acetyl group in a reaction catalysed by the acetylcholinesterase (Voet and Voet, 1995). Acetylcholine is a white crystalline compound released from the ends of nerve fibers and is involved in the transmission of nerve impulses. Acetylcholinesterase is an enzyme, present in the blood and some nerve endings that aids the breakdown of acetylcholine and supresses its stimulating effects on nerves. *Calotropis procera* leaves had about 78% activities which is even higher than the catechin, standard . This shows that CPL can be studied for use in the treatment of brain diseases

4. Conclusion

The extracts of *Calotropisprocera* present anti-acetylcholinesterasic activities which may render them potentially useful as an adjuvant in the treatment of cognitive diseases such as Alzheimer's disease. The activities reported in the present work show CPL may contribute to increase the levels of acetylcholine in cholinergic neurons. Our results clearly show that CPL had the strong anti-acetylcholinesterasic activity.

5. Future Scope

Calotropisprocera leaves can be channel into drug design, which can be used in the treatment of Alzheimers disease and other neurodegenerative diseases. It can also be further for use in the treatment of brain diseases.

6. Acknowledgement

I want to say a big thank you to Pastor (Dr.) & Mrs O.A Adaramoye and Mr & Mrs L.A Odeja Olajuyin who sponsored this research.

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