

Figure 2 and Figure 3: Mechanism of Ache Action In Neurotransmission and Selected Reversible Ache Inhibitors in Pharmacotherapy of Ad; Source:Arceet *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 (Mhaskaret *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 to 18cm 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 nematocides, 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 acetylcholinesterase activity and lipid peroxidation. Hence, the acetylcholinesterase activity and lipid peroxidation of the methanol extract of *Calotropisprocera* was investigated.

2. Material and Methods

2.1 Plant 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 12H₂O, Tris Buffer, Sodium di-hydrogen phosphate 2H₂O 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,

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

Where A_{control} is the absorbance of the control, and A_{sample} 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 $\mu\text{moles}/\text{min}/\text{g}$ tissue.

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

Where A, B, C, D & E are Δ absorbance from 2mins to 10mins.

Enzyme Activity = %Rate

Percentage Inhibition = 100 - % enzyme activity

3. Results and Discussion

3.1 Lipid Peroxidation of *Calotropisprocera*

The lipid peroxides scavenging activities of *Calotropisprocera* leaves in rat liver homogenates was given in Tables 1. At concentration of 250- 1000 $\mu\text{g}/\text{ml}$, the scavenging activities of *Calotropisprocera* 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 *Calotropisprocera* leaves in rat liver was statistically higher than Catechin

The lipid peroxides scavenging activities of *Calotropisprocera* leaves in rat brain homogenates was given in Tables 2. At concentration of 750- 1000 $\mu\text{g}/\text{ml}$, the scavenging activities of *Calotropisprocera* 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 *Calotropisprocera* 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 *Calotropisprocera* was compared with catechin (standard). The methanolic extracts of CAL had higher activity than that of catechin. At the concentration of 10-750 $\mu\text{g}/\text{ml}$, 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 *Calotropisprocera* leaves Extracts On Iron/Ascorbate- Induced Lipid Peroxidation (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 \pm 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 *Calotropisprocera* Leaves Extracts On Iron/Ascorbate- Induced Lipid Peroxidation (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 \pm 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 \pm SD (n=4),* Significantly different from control ($p < 0.05$) CPL; *Calotropis procera* leaves. Acetylcholine is involved in the signal transfer in the synapses. After been delivered in the synapses, acetylcholine is hydrolysed 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 suppresses 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 *Calotropis procera* present anti-acetylcholinesterase 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-acetylcholinesterase activity.

5. Future Scope

Calotropis procera leaves can be channel into drug design, which can be used in the treatment of Alzheimer's disease and other neurodegenerative diseases. It can also be further for use in the treatment of brain diseases.

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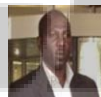
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