Antiepileptic/Anti-convulsant Effects of Ocimum basilicum Smoke in Albino Rabbits

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ABSTRACT

The ethanol, chloroform and whole-leaf extracts of Ocimum basHicum were evaluated for their anti-epileptic/anti-convulsant effects on rabbits. Convulsion was induced in these animals with over-dosage of penicillin. They were exposed to the smokes of the different extracts as they were having convulsive feasts. The concentrations of glucose, creatinine and calcium in their sera were measured spectrophotometrically before, during and after convulsion. The mean calculated concentrations in the control before during and after convulsion were glucose: 107.50 ± 46.70mg/dl, creatinine: 1.55 ± 2.19mg/dl, and calcium: 9.00 ± 5.66mg/dl respectively. The mean calculated concentrations in those exposed to chloroform extract smoke before, during and after convulsion were glucose: 57.90 ± 6.61mg/dl, creatinine: 3.13 ± 1.19mg/dl, and calcium: 11.70 ± 1.56mg/dl respectively. The mean calculated concentrations in those exposed to ethanol extract smoke before, during and after convulsion were glucose: 63.67 ± 26.21 mg/dl, creatinine: 2.70 ± 0.70mg/dl and calcium: 11.67 ± 2.50mg/dl respectively. And the mean calculated concentrations in those exposed to whole-leaf extract smoke before, during and after convulsion were glucose: 84.40 ± 32.70mg/dl, creatinine: 3.10 ± 1.49mg/dl and calcium: 11.33 ± 3.25mg/dl respectively. The
results showed that the individual extracts of *Ocimum basilicum* had low anti-epileptic/anti-convulsive effects. The whole leaf extract had a profound effect when the animals were exposed to its smoke. This suggests that the components of the extracts act in synergy to give the observed effect.

**KEYWORDS:** *Ocimum basilicum*, whole leaf extract, anti-epileptic/anti-convulsant effects on rabbits.

**INTRODUCTION**

Few experiences match the drama of a convulsive seizure. A person having a severe seizure may cry out, fall to the floor unconscious, twitch or move uncontrollably, drool and even have "Black outs" or periods of confused memory (Glasser, 1989). Within minutes, the attack is over and the person regains consciousness but is exhausted and dazed. This is the image most people have when they hear the word "Epilepsy" (Schwartzkroin, 1993). However, this type of seizure is one type of epilepsy.

Seizure is an alternative term for "epileptic attack" (Goddard, 1999). Based on the type of behaviour and brain activity, seizures are divided into two broad categories: generalized and partial (also called local or focal) (Reynolds, 1990). In partial seizures, abnormal electrics! discharge originates from one specific area of the brain, and in generalized seizures, the whole brain is involved (Aminoff and Parent, 1997). Partial seizures are divided into simple and complex types. Simple partial seizures are those in which the epileptic activity in one area of the brain does not interfere with consciousness. Thus, a person whose epilepsy has been caused by injury to the area of the brain controls movements of one leg may experience a series of involuntary jerking movements of that leg as the only symptom (Chauvel, 1994). Complex partial seizures involve some alteration of awareness. The commonest example is where the discharge originates from one of the temporal lobes of the brain. In this case, the attack may consist of a feeling of intense familiarity with the surroundings but being unable to respond. Also, automatic chomping movements of the jaw may occur (Verity et al., 1991).

Generalized seizures are a result of abnormal neuronal activity on both sides of the brain. These seizures may cause loss of consciousness, falls, or massive muscle spasms (Aminoff and Parent, 1997). There are many kinds of generalized seizures. In *absence seizures*, the person may appear to be staring into space and have jerking or twitching muscles. These seizures are sometimes referred to as petit mal seizures, which is an older term. *Tonic*
seizures} cause stiffening of muscles of the body, generally those in the back, legs, and arms (Goddard, 1999). Cleric seizures cause repeated jerking movements of muscles on both sides of the body. Myoclonic seizures cause jerks or twitches of the upper body, arms, or legs (Aicardi, 1994). Atonic seizures cause a loss of normal muscle tone. Tonic-clonic seizures cause a mixture of symptoms, including stiffening of the body and repeated jerks of the arms and legs as well as loss of consciousness. Tonic-clonic seizures are sometimes referred to by an older term: grand mal seizure (Arthur and John, 1996). Statistics on ground make it clear that improved treatments are desperately needed. However, current available treatments that control seizures are; drug therapy; this focuses on reducing the frequency and severity of seizures and the type of seizures experienced by the patient. Commonly prescribed drugs include benzodiazepines such as clonazepam, clorazepate and diazepam as well as phenytoin, lamotrigine, carbamazepine, valproic acid and Phenobarbital (Aminoff snd Parent, 1997). Also surgery and devices such as vagus nerve stimulator implanted under the skin of the chest and attached to vagus nerve in the lower neck cap’ be applied to manage seizures (Chauvel, 1994). In addition, dietary approach has also been used based on the observation that ketosis is associated with reduction of seizures. Also, nutritional supplements such as vitamin E (for children), folic acid, melatonin, taurine and vitamin B₆ had been used in alleviating epileptic seizures in patients (Freeman et aL., 1994).

Many medicinal plants have been used to cure various kinds of ailments by traditional medicine practitioners. For example, Garlic (Alhuthum sativum), in the reduction of high blood pressure, African cucumber (Momorbica charantia) as an abortifacient, Chinese herb (bupleurum and Asian ginseng root) have been shown to be helpful to epileptic patients (Narita et al., 1982). As well as Ocimum basilicum (basil plant) in reducing fever, antispasmodic, analgesic, anti-inflammatory e.t.c. (Simon et al., 1990). In addition to Ocimum basilicum efficacy, its smoke is used to cure and control epilepsy by traditional medicine practitioners in village settings.

AIMS AND OBJECTIVES
The aims and objectives of this project work is to ascertain the efficacy of Ocimum basilicum smoke on epileptic patients in accordance with specific diagnostic parameters using albino rabbits.
MATERIALS AND METHODS

CHEMICAL MATERIALS
The following are chemicals utilized: Ethanol, chloroform, sodium hydroxide, picric acid, sodium tungstate, tetraoxsulphate (vi) acid, distilled water, standard calcium solution, standard creatinine solution, ammonium oxalate, ammonium solution, potassium permanganate, fluoride oxalate, Glucose test kit (Bronila Diagnostic Test combinations- CAT.NO.2004).

PHYSICAL MATERIALS
Physical materials used are as follows:
Whatman filter paper, local mortar and pistle, kitchen knife, plastic buckets, measuring cylinder, conical flasks, beakers, wire gauze, retort stand, Gas cylinder, matches/ lighter, syringe needles, wrist watch, perforated cages, Rubber pipe, swab handglooves, test tubes, masking tapes, Bunsen burner, burrette tripod stand, test tubes racks.

BIOLOGICAL MATERIALS
The biological materials utilized are albino rabbits and Ocimum basilicum (Basil plant) leaves.

INSTRUMENTS /EQUIPMENT

PREPARATION OF EXTRACTS
The basil plant leaves was obtained from Meat Market Abakaliki,Ebonyi state. The leaves were cut into pieces before being pounded in a mortar for 45 minutes. The leaves weighed 350g/kg for three different extracts. Two of the 350g/kg leaves was immersed in 500ml ethanol and 500ml chloroform respectively and allowed for 24hours before filtration with other remaining as raw extract. The three extracts was placed in an electric oven for drying at normal room temperature (25°C). After drying each weighed 43.8g/kg, 40.2g/kg, 51.1g/kg for ethanol, chloroform, and raw extract respectively.

SAMPLE COLLECTION
The albino rabbits used in this project research was bought from Pharmacy department University of Nigeria Nsukka, Enugu State. Each of the rabbits was 3 months old.
PREPARATION OF DRUG
The penicillin was bought from Cino pharmacy stores, Zik Avenue Abakaliki, Ebonyi State. It was manufactured by Ajembic Limited, India, for HELM PHARMACEUTICALS GMBH with NAFDAC reg. No. 04-0891. The drug was prepared by mixing 10ml of 0.1N normal saline with the drug and kept in a refrigerator.

SAMPLE PROCESSING
1.30kg and 1.15kg and grouped into AA, AB, AC, X and K respectively. Where group X served as negative control and group K as positive control.

Four groups were induced with penicillin injection intramuscularly with dose range in accordance with their body weight. They were observed for about 5 to 7 minutes, before administration of different basil plant extracts smoke.

Procedure: group AA in perforated cage were exposed to 5.0g/kg, 15.0g/kg and 20.0g/kg chloroform extract smoke for 120 seconds for 3 consecutive days. Group AB in perforated cage were exposed to 5.0g/kg, 5.0g/kg and 20.0g/kg ethanol extract smoke for 120 seconds for 3 consecutive days.

Group AC in perforated cage were exposed to 5.0g/kg, 15.0g/kg and 20.0g/kg raw extract smoke for 120 seconds for 3 consecutive days.
Group X were untreated after inducement
Group K were uninduced.

METHODS
CREATININE ESTIMATION (JAFFS METHOD)
Principle
Serum is diluted with distilled water and the proteins are precipitated with tungstic acid. Creatinine in the supernatant reacts with hydroxyl ion (OH") of picrate solution to form a yellow - red coloured complex, which is estimated spectrophotometrically at 500nm. The intensity of the colour is Proportional to creatinine concentration (Monica, 2002).

Materials
Plasma, 5% sodium tungstate, 0.33M H₂SO₄, 0.75M NaOH, standard creatinine solution 20mg/dl Procedure Pipette into centrifuge tube in
<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5% sodium tungstate</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.33M H₂SO₄</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

These were allowed to stand for 5 minutes for concrete precipitation and later centrifuged for 5 minutes.

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant (T)</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Creatinine standard (5%)</td>
<td>-</td>
<td>3.0</td>
<td>' • &quot;'</td>
</tr>
<tr>
<td>Picric acid</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>0.75 NaOH</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* These were left for 15 minutes and the absorbance read at 500nm.

**Calculation**

Abs (AE) of test x 3.0ml = Mg creatinine/dl

Abs (AE) of standard AE = O.D SOsecs - O.D 20secs where O.D SOsecs = Absorbance after SOsecs O.D 20secs = Absorbance after 20secs

AET = the difference between absorbance of test at SOsecs and 20secs. AES = the difference between absorbance of standard at SOsecs and 20secs.

* Normal value: 0.5 to 2.5 mg/dl. (Mitrika, 1977)*

**3.5.2 Glucose Estimation (Nelson's Method) Principle**

Glucose reduces alkaline copper reagent to cuprous oxide in the presence of heat. The cuprous oxide then reduces arsenomolybdate to molybdenum blue colour that is read spectrophotometrically (Monica, 2002).

**Materials**

Glucose test kit reagents, distilled water, test tubes, beaker, spectrophotometer, fluoride oxalate.

**Procedure**

Pipette into centrifuge tube in mis

<table>
<thead>
<tr>
<th></th>
<th>Test (T)</th>
<th>Std (S)</th>
<th>Rₜ blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution 1</td>
<td>1.85</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sample serum</td>
<td>0.05</td>
<td>-</td>
<td>(mix)</td>
</tr>
<tr>
<td>Solution 2</td>
<td>0.05</td>
<td>-</td>
<td>(mix)</td>
</tr>
</tbody>
</table>

These were flowed to centrifugor 5 mins.
**CALCIUM ESTIMATION**

**Principle**

The calcium in heparinized plasma is precipitated as calcium oxalate. After washing the precipitate thoroughly, it is dissolved in hot H$_2$SO$_4$ and titrated hot with standard permanganate (Monica, 2002).

**Materials**

Plasma, 100mg/dm$^3$ standard calcium solution, 4% ammonium oxalate, 0.5M H$_2$SO$_4$, 2% ammonium solution, filter paper, 0.01 M KmnO$_4$ and general glassware.

**Procedure**

Pipette into test tubes in mis.

<table>
<thead>
<tr>
<th></th>
<th>Test (T)</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Std calcium solution(10 mg/cm)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Distilled water</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>4% ammonium oxalate</td>
<td>2.0</td>
<td>2.0   (mix)</td>
</tr>
</tbody>
</table>

These were allowed to stand for 1 hour, shaking at frequent interval. The mixture was filtered and precipitate washed twice (x2) with 3.0ml portions of 2% ammonia solution. The filter paper and the precipitate were carefully transferred into 250cm$^3$ conical flask and 10ml of 0.5m H$_2$SO$_4$ and some distilled water added and heated to almost boiling. The hot solution and the filter paper was titrated with 0.01mKMn0$_4$ to a faint pink end point. The titration was repeated using blank containing 10ml 0.5mH$_2$SO$_4$ and 50ml distilled water.

* Read absorbance of sample (T) and standard (S) against the reagent blank (RB).

A (T) x 120mg/100ml = Mg Glucose/100ml,

A (S)

Or

A (T) x 6.66mmol/L = mmol Glucose/L

A (S)

Normal values: 75 to 140mg/di. (Mitraka, 1977).
Burette Readings for:
Test total
Titre value Blank

<p>| | | |</p>
<table>
<thead>
<tr>
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<th></th>
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<tbody>
<tr>
<td>Final</td>
<td></td>
<td></td>
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<tr>
<td>Initial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Titre value =
Calculation:
1ml 0.01M KmnO₄ = 0.2mg calcium
since 2.0ml of plasma was used,Mg calcium per 100cm³ plasma = (titre of test - blank) x 0.2 x 100
1 2
Normal value: 5.5 to 12.6mg/dl. (Mitruka, 1977).

RESULTS
4.1.1 EXTRACTION YIELD

Table 4.1: Per Yield of Extraction of Ocimum Basilicum Leaves

<table>
<thead>
<tr>
<th>Type of solvent</th>
<th>Initial mass of leaves before extraction (g/kg)</th>
<th>Mass of leaves after extraction (9/kg)</th>
<th>Percentage extraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>350</td>
<td>43.80</td>
<td>12.5</td>
</tr>
<tr>
<td>Chloroform</td>
<td>350</td>
<td>40.20</td>
<td>11.5</td>
</tr>
<tr>
<td>-</td>
<td>350</td>
<td>51.10</td>
<td>14.6</td>
</tr>
</tbody>
</table>

The percentage yield of extraction of fresh and dry leaves of Ocimum basilicum using ethanol, chloroform solvents and non-solvent extract (whole leaf or raw) gave rise to 12.5%, 11.5% and 14.6% respectively (TABLE 4.1).

PHYSICAL OBSERVATION

During the course of administration of the extracts after inducement of the animals with penicilllin, the animals exposed to chloroform extract smoke were observed to be weaker, calm and sluggish in movement. Also after two days of the experiment one of the animals exposed to chloroform extract smoke died. The animals exposed to ethanoli extract smoke were found to be slightly weak, reduced activity and sluggish in movement. The animals exposed to raw extract smoke were found to be mildly active, and slow in movement while those that were not exposed to any extract smoke continued to be active before, during and after the experiment.
Table 4.2; Average Concentrations of Glucose, Creatinine and Calcium Levels In The Serum And Plasma Of Different Rabbits After 3 Days Of Administration

<table>
<thead>
<tr>
<th>Groups A.</th>
<th>Glucose (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Calcium (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Those expose</td>
<td>57.90 ± 6.61</td>
<td>3.13±1.19</td>
<td>11.70 ± 1.56</td>
</tr>
</tbody>
</table>

Those exposed to chloroform extract smoke (GRPAA)

<table>
<thead>
<tr>
<th></th>
<th>Glucose (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Calcium (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Those exposed to ethanol extract smoke (GRP AB)</td>
<td>63.67 ±26.21</td>
<td>2. 70 ±0.70</td>
<td>11. 67 ±2.50</td>
</tr>
<tr>
<td>Those exposed to raw extract smoke (GRPAC)</td>
<td>84.40 ±32.70</td>
<td>3.10±1.49</td>
<td>11.33 ±3.25</td>
</tr>
<tr>
<td>Those not exposed to any extract smoke (GRP K)</td>
<td>107.50 ±46.70</td>
<td>1.55±2.19</td>
<td>9.00±5.66</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation

Results were considered significant at P < 0.05.

After three days of administration of extracts, the mean concentration of glucose in animals exposed to chloroform extract smoke, ethanol extract smoke and raw extract smoke (57.90, 63.67, 84.40 mg/dl's respectively) were found to be lower than those not exposed to any extract smoke (107.50mg/dl) which is the positive control experiment.

Also, the mean concentration of creatinine in animals exposed to chloroform, ethanol and raw extract smokes (3.13mg/dl, 2.70mg/dl, and 3.10 mg/dl) respectively were found to be higher than those not exposed to any extract smoke (1.55mg/dl).

Lastly, the mean concentration of total calcium ions in animals exposed to chloroform, ethanol and raw extract samples (11.70mg/dl, 11.67mg/dl, and 11.33mg/dl) respectively were found to be slight higher than those animals not exposed to any extract smoke (9.00mg/dl).
Figure 4.1: Graphical Representation of Total Calcium Concentration Against Three Days Of Administration Key

@- GRPAA
x - GRP AB
O- GRPAC

15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0

0
i

Days of administration

"Figure 4.1: Shows Graphical Representation of total Calcium ion concentration against three days of administration"

After three days of administration of extracts, the mean concentration of total calcium ions in animals exposed to chloroform extract smoke (grp AA) and ethanoi extract smoke (grp AB) were slightly higher than those exposed to whole -leaf (raw) extract smoke.
Figure 4.2: Graphical Representation of Glucose Concentration Against Three Days of Administration

Key

- GRPAA
- GRPAB
- GRPAC

15C 140
130
120

Days of administration

Figure 4.2: Shows Graphical Representation of Glucose
Concentrations against three days of administration

After three days of administration of extracts, the mean concentration of glucose in animals exposed to chloroform extract smoke (grp AA) and ethanol extract smoke (grp AB) were lower than those exposed to whole-leaf (raw) extract smoke.

Figure. 4.3: Graphical Representation of Creatinine Concentration Against Three Days Of Administration

Key

<table>
<thead>
<tr>
<th>Group</th>
<th>Key</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>GRPAA</td>
</tr>
<tr>
<td>AB</td>
<td>GRPAB</td>
</tr>
<tr>
<td>AC</td>
<td>GRPAC</td>
</tr>
</tbody>
</table>

6.0

5.0

Days of administration

Figure 4.3: Shows Graphical Representation of creatinine concentration against three days of administration

After three days of administration of extracts, the mean concentration of creatinine in animals exposed to chloroform extract smoke Group AA) and ethanol extract smoke (Group AB) were slightly higher than those exposed to whole-leaf (raw) extract smoke.

The entire graphs above illustrate that animals in group AC responds proportionately to normal than animals in group AB and A.

DISCUSSION

The extraction of fresh leaves of *Ocimum basilicum* with ethanol, chloroform yielded 12.5%, 11.5% respectively. This low percentage in comparison with raw extract 14.6% suggests that most of the chemical component of the leaves had low solubility in ethanol and chloroform. This may also suggest that the solvents especially chloroform penetrate the blood-brain barrier at relatively faster rate than ethanol. This finding agrees with the findings of Ernst et al. (2003) that perturbations on the integrity of the brain resulted to focal disruption of the...
blood-brain barrier in the rat cortex by direct application of halogenated alkane compounds and bile salts and suggest that the extracts may contain halogenated compounds. This may be used to explain the death of one of the animals exposed to chloroform extract smoke in the experiment. However, the effect of chloroform extract may be due to the loss of the very volatile active ingredients in the extract before administration to the animals.

In the inducement of the animals with penicillin, the animals were observed to be weak, calm and slow in movement. This suggests impairment of neurotransmission in these animals through the effect of the drug on the inhibition of a post-synaptic neurotransmitter like GABA and increase in the synthesis of excitatory amino acid like glutamate. This observation was in accordance with Schneiderman and Evans (1986), which suggested that penicillin enhances seizure susceptibility in rabbits by increasing the level of glutamate in the cortex and brain stem.

In the administration of the extracts, the animals exposed to chloroform and ethanol extracts smoke (groups AA and AB) were weaker and slower in movement than those that were exposed to raw extract smoke (group AC). The reduction in appetite could account for their reduced activity unlike those exposed to whole-leaf (raw) extract smoke (group AC) and those not exposed to any extract smoke (group K). The actual mechanism behind the reduced physical activities of the rabbits treated with ethanol and chloroform extracts is not clearly understood. This might occur as a result of the effect of the organic extract of *Ocimum basilicum* smoke on the animals during the course of treatment. This agrees with the observation made by Agbafor (1999) given an organic extract of Baphia nilida to albino rats. The difference between the glucose levels in the animals treated with the smokes of the extracts and those that served as the control was significant. Also there was significant difference (P>0.05) between glucose levels in the animals exposed to chloroform, ethanol and raw extracts smoke. This difference in the average level of glucose may suggest that organic extract contains some inhibitors, which tend to inhibit some enzymes of the glycolytic pathway. This finding is in accordance with observations Phippen and Simon (1998) which suggest that phytochemically, *Ocimum basilicum* contains potentially toxic compounds like safrole, rutin, caffeic acid and quercetin.

The average levels of creatinine after administration of extracts to groups was significantly lower (P>0.05) than those that were not exposed to any extract smoke. Also, there was a slight significant difference (P>0.05) in animals exposed to chloroform, ethanol and raw
extracts smoke. The mechanism of this action cannot be explained vividly but may occur as a result of decrease in food and water intake after the first day of administration of the extracts due to hypothalamic inhibition. Also, the difference of lower levels of creatinine observed in the animals exposed to ethanol, chloroform and raw extracts might be due to reduction in the rate of release of metabolic fuels such as glucose and fatty acids. This finding agreed in accordance with Cohen (2002) which suggest that slight increase in creatinine levels may occur as a result of dehydration and stimulation of other sources of ATP to the animal like tricarboxylic acid, pentose phosphate pathways. The difference between the total calcium ion levels in animals treated with the extracts and those that served as the control was significant. Also there was significant difference (P>0.05) between calcium ion levels in the animals exposed to chloroform, ethanol and raw extracts smoke. This difference in the average level of calcium ions may suggest that raw extract contains some inhibitors which tend to inhibit calcium ion influx in skeletal muscle and extracellular fluid, and agrees with the findings of Brett and Michael (1999) that *Ocimum basilicum* contains essential compounds like dl-limonene, eugenol, methyl-cinnamate, camphor, methylchavicol that can block influx of calcium ions into the muscle cells and this may be used to explain the observation made on animals that were exposed to raw extract smokes in the test.

**CONCLUSION**

In conclusion, individual extracts of *Ocimum basilicum* had low anti - epileptic/anti - convulsive effects. The raw extract had a profound effect when the animals were exposed to its smoke. This suggests that the components of the extracts act in synergy to give the observed effect.

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