DETERMINATION OF ANTICONVULSANT ACTIVITY OF METHANOL LEAVES EXTRACT OF *CARICA PAPAYA* USING MICE AND CHICKS

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Abstract:

Epilepsy is a chronic neurological disorder of the brain function that is characterized by a periodic and unpredictable occurrence of seizures. *Carica papaya* infusion has been used to treat epilepsy by traditional medicine practitioners in many communities of Kaduna State, Nigeria. Present work aimed to evaluate the anticonvulsant effects produced by acute administration of 70% methanol leaves extract of *Carica papaya* (CPLE) using two experimental models: Pentylenetetrazole-induced seizures (PTZ) and Maximal Electroshock-induced seizures (MES) in mice and chicks respectively. Phenytoin 20mg/kg and Valproate 200mg/kg body weight were used as standard drugs for MES seizures and PTZ induced seizures respectively. In MES induced seizures, CPLE increased the time of onset of Tonic Hind Limb Extension (THLE) at doses of 200 and 400mg/kg body weight. Extract caused an insignificant decrease ($p > 0.05$)in duration of THLE. Percentage protection of MES seizures with dose of 400 mg/kg body weight was 100%. In PTZ induced convulsion, CPLE at 400 mg/kg dose significantly increased ($p \leq 0.05$) mean latency period. Percentage protection of PTZ induced seizures at 200 and 400 mg/kg doses were 60% and 80% respectively, which implied a dose-dependent activity. Phenytoin in MES-induced seizures and Valproate in PTZ induced seizures produced 100% seizure protection. CPLE suppressed seizures induced by MES, however a significant difference ($p \leq 0.05$) was observed in PTZ-induced seizure. The LD_{50} of CPLE was ≥ 2000 mg/kg body weight. Preliminary phytochemical screening showed presence of flavonoids, Triterpenes, Saponins and Alkaloids. Results therefore, provided evidence of anticonvulsant effects by CPLE. Hence, CPLE may be developed as a safe and cheaper alternative drug for long-term use in therapeutic management of neurological disorders characterized by convulsions.

Keywords: Anti-convulsion, *Carica papaya*, Epilepsy, Neurological disorders

Introduction:

Epilepsy is a term used to describe a group of neurological disorders, all of which exhibit periodic seizures (Rang *et al*., 2012**)**. Seizure refers to a transient alteration of behaviour due to the disordered, synchronous and rhythmic firing of populations of brain neurons. Epilepsy is therefore, a disorder of the brain function characterized by a periodic and unpredictable occurrence of seizures (Goodman and Gilman, 2011). As the second most chronic neurological disorder, epilepsy has no age, racial, social, sexual or geographical boundaries. Often, there is no recognisable cause, although it may develop after brain damage, such as trauma, stroke, infection or tumour growth or other kinds of neurological diseases, including various inherited neurological syndromes (Rang *et al*, 2012.

The lifetime prevalence of the disease in the general population ranges from 2.3 to 15.9 per 1,000 in high-income countries and from 3.6 to 15.4 per 1,000 in low-income countries (Bell *et al*., 2014). At a conservative estimate, 50 million people worldwide have epilepsy with an annual incidence ranging from 20 to 70 cases per 100,000 and point prevalence of 0.4 to 0.8 percent (Ogunrin 2006). The incidence rates are highest in childhood, plateaus from the age of 15 to 65 years, and rise again among the elderly (Ogunrin, 2006). About 10% of the whole world population living a normal life span can expect to have at least one epileptic seizure (Engel, 2002).

Herbal medicines are the oldest form of medical treatment in human history. Plants are said to form the main ingredient of medicine in traditional system of healing and have been sources of inspiration for several major pharmaceutical drugs. Most of the commonly used drugs today are of herbal origin and about 25% of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material.

The World Health Organization (WHO, 2006) estimated that 4 billion people, representing 80% of the world population, presently use some herbal medicine for some aspect of primary healthcare. Plant extract can be an important source of natural and safer drugs for treatment of Epilepsy. According to World Health Organization, 80% of the population have no access to primary health care, because they live far away from health centres or lack resources to purchase prescription drugs (WHO, 2004).

One of the important areas in which herbalism enjoys high patronage is in the management of neurological and psychiatric disorders (Chindo, 2018). Herbal medicines have been reasonable alternatives for the management of mental disorders such as anxiety, depression and dementia among many others. Several medicinal plants have shown potentials for use as anticonvulsants. For example Musa *et al.,* (2006) reported strong anticonvulsant effect of *Ficus thoningii* Blums. In the search for new therapeutic agents for the treatment of neurological disorders, medicinal plant research has made a lot of contributions by demonstrating pharmacological effectiveness of different herbs in various animal models. The development of anti-epileptics from herbal sources, seem to be a reasonable approach due to their therapeutic efficacy and lower incidence of side effects. It is believed that plants of Boraginaceae family are rich of fatty

acids, especially gamma linoleic acid and flavonoids. There are some evidences about anticonvulsant effect of this fatty acid and some flavonoid compounds. The anti-seizure effect of *E.amoenum Fisch* may be related in part to linoleic acid and/or flavonoid compounds present in the extract (Heidari *et al.*, 2006).

Epilepsy is strongly associated with significant Psychological and social consequences of everyday living. Some of the problems encountered with pharmacotherapy of epilepsy, include economic and social burden of drug treatment, patient compliance, side effects of the current medication used and lack of availability of the drugs in rural areas in a developing nation like Nigeria.

As majority of antiepileptic drugs are consumed for life, concomitant administration of other drugs predisposes to the risk of drug interaction. Despite the availability of several AEDs in urban areas, the treatment of epilepsy is still far from adequate. Several anti-seizure drugs are available clinically and in areas of research, however there is need to further improve prognosis and ultimately, quality of life of patients living with epilepsy (Bertram, *et al.*, 2012).

Thus there is still the need to develop new drugs with greater clinical efficacy, tolerability, minimal side effect, devoid of unfavourable drug interactions and better pharmacokinetic properties.

This study seeks to verify claims by traditional medicine practitioners of the possible anticonvulsant activity of *Carica papaya* leaves extract.

Materials and Methods

Materials:

Phenytoin Sodium capsules (Norris Medicines Limited, India), Sodium Valproate (ASSOS, Turkey), Pentylenetetrazole(Carl Roth, Karlsruhe, Germany), Methanol extract of *Carica papaya* at doses of 100, 200 and 400 mg/kg body weight.

Methods:

Plant collection and Identification:

Carica papaya leaves were collected in July 2018 from Rimi College Staff quarters, Unguwan Rimi, Kaduna. The leaves were identified and authenticated by Mallam U.S Gallah and a specimen with voucher number 1764 was deposited at the Herbarium of Department of Biological Sciences, Kaduna State University.

Extraction:

The leaves collected were air-dried under shade, then powdered mechanically using mortar and pestle. 620 g of powdered leaves was extracted with 2.1 L of 70% v/v of methanol, for three days using maceration technique with occasional stirring. The liquid was then strained off and the solid residue (marc) was pressed to remove the solution as much as possible. Filtering was carried out using a clean cloth and then with Whatman filter papers. The extract was concentrated in a Rotary evaporator (78.8^{o} C) for 6 hours and the concentrated extract was finally dried in an oven for 5 days at temperature of 50^{o} C. The dried extract was weighed, then kept at 4° C in a refrigerator in an amber air-tight container until use. The percentage yield was calculated.

Animals:

Healthy adult Swiss Albino male and female mice (16-28 g) and white ranger cockerels (chicks) weighing (34-55 g) obtained from the Animal House, Department of Pharmacology and Toxicology Kaduna State University and Animal house Department of Pharmacology and Toxicology Ahmadu Bello University, Zaria respectively, were used for the study. All animals were kept in clean dry cages maintained in a well-ventilated animal house at a controlled temperature (25 ± 1° C) and a 12-h dark/light cycle. Animals had free access to water and food *ad libitum*. All tests were conducted in quiet rooms at the same controlled conditions above and conformed to the principle for research involving animals.

Ethical Approval:

Permission and approval for animal studies were obtained from the Kaduna State University Animal Ethics Committee.

Phytochemical Screening:

Phytochemical screening of extract was carried out at the Department of Pharmacognosy and Drugs Development, Kaduna State University, Kaduna using the method described by Trease and Evans (2008). The Extract was screened for the presence or absence of alkaloids, flavonoids, saponins, tannins, glycosides, steroids, triterpenes, anthraquinones and carbohydrates.

Acute Toxicity Test:

Test was carried out using the OECD 425 method (2008). Six adult Swiss albino mice (19-21 g) were used and dose used for the limit test was 5000 mg/kg body weight. Test was carried out using both oral and interperitonial (I.P) routes of administration. Two mice were starved of food and water for 3 hours, after which one mouse, was administered the extract at dose of 5000 mg/kg of body weight orally, while the other was administered same dose interperitoneally. They were observed for signs of toxicity (sedation, sniffing, grooming and rearing) and mortality for 24hours. Four mice were divided into two groups of two mice each. The first group was administered 5000 mg/kg body weight of the extract orally while the second group was administered 2000 mg/kg body weight of the extract intraperitoneally. Both groups were observed for signs of toxicity within 24 hours. Absence of mortality led to termination of test and all tested mice were observed for 14days.

Evaluation of Anticonvulsant activity

Pentylenetetrazole-induced Seizures

Method of (Swinyard *et al.*, 1989) and (Raza *et al.*, 2001) were employed. Twenty five adult male and female Swiss albino mice (16-28 g) were randomly divided into five groups of five mice each. The first, second and third groups were administered 100, 200, 400 mg/kg body weight of the extract (i.p) respectively. Fourth group (positive control) were administered 200 mg/kg body weight of Sodium Valproate (i.p). Fifth group (negative control) were administered 0.9 % Normal saline (10 ml/kg body weight) interperitonially. After thirty minutes post treatment, all the groups were given Pentylenetetrazole 85 mg/kg body weight interperitoneally and observed over a period of 30 minutes. The observed and recorded parameters were: onset of seizures and duration of convulsion. Absence of an episode of clonic seizures of at least 5 seconds indicated the ability of the extract to abolish effect of Pentylenetetrazole on seizure threshold.

Maximal Electroshock Test

Methods of Swinyard and Kupferberg (1985) and of Browning (1992) were employed. Twenty five cockerel chicks were divided into five groups of five chicks each. The first, second and third groups were injected (i.p) with 100, 200 and 400 mg/kg body weight of the extract respectively. Group four chicks (positive control) were injected (i.p) with Phenytoin 20mg/kg body weight. Group five (negative control group) were injected with 0.9% Normal saline at 10 ml/kg body weight (i.p). Each administration was given thirty minutes before the Electro Shock Test. After 30 minutes post treatment, maximal electric shock was delivered to the chicks to induce seizure using Ugobasile electro-convulsant machine, with corneal electrodes placed on the upper eyelids of the chicks. A current of 80MA (which produced a tonic seizure of the hind limb in 100% of the chicks) was used throughout the study. The onset and duration of convulsion were observed and recorded. The ability to prevent or shorten the recovery time from tonic hind limb extension was considered an indication of anticonvulsant activity.

Statistical analysis

All quantitative data were presented as mean ± standard error of mean (SEM). Results were analysed using one way ANOVA, followed by Dunnett´s Post Hoc and Tukey HDS. Significant differences between means were assessed at 95% level of significance i.e. $p < 0.05$ were considered significant.

Results and Discussion

The percentage yield obtained was 13.23%. Preliminary Phytochemical tests showed the extract tested positive to Alkaloids, Carbohydrates, Anthraquinones, Steroids, Flavonoids, Glycosides, Saponin and Tannins and negative to combine Anthracene derivative. Previous studies revealed that the anticonvulsant activity of herbal drugs is due to presence of different chemical constituents like Alkaloids, Flavanoids, Triterpenoids and Saponins (Mehdipour *et al*., 2006). Flavonoids have a broad spectrum of biological activities including anti-bacterial, anti-oxidant, anti-diabetic, anti-cancer, cardiovascular, analgesic, anti-inflammatory, anti-convulsant, anti-anxiety etc (Sidana *et al.*, 2013). Previous study on plants containing flavonoids and linoleic acid such as *Echium amoenum* (Heidari *et al*., 2006), monoterpenes and Triterpenoids such as *Ferula gummosa* (Sayyad *et al.*, 2003) were found to possess anticonvulsant activity. Also previous study on *Ficus platyphylla* demonstrated anticonvulsant properties of saponins (Chindo *et al*., 2008).

Result of acute toxicity study showed that LD_{50} was greater than 2000 mg/kg body weight. Signs of toxicity observed were: grooming, sedation, sniffing and rearing. The LD_{50} after both oral and i.p administration implied that the extract was practically non-toxic. LD_{50} is a way of measuring the acute or short-term toxic potential of a substance and thus, its safety margin. The absence of death recorded in the acute toxicity study was therefore an indication of how safe the plant is at the doses used.

In the PTZ-induced convulsions, extract at doses of 100, 200 and 400 mg/kg delayed the onset of seizure from 1.45 ± 0.17 to 2.78 ± 0.5, 3.06 ± 0.54 and 4.15 ± 0.59 respectively (Fig. 1). Also, the extract at doses of 100, 200, and 400 mg/kg shortened the recovery time from 23.49 ± 2.02 to 18.65 ± 6.87, 16.67 ± 0.71 and 14.30 ± 0.63 respectively. The result at 400 mg/ kg body weight was significant at $p<0.05$ for onset and duration of seizure. Sodium Valproate shortened the recovery time and showed 100% protection (Fig. 2).

PTZ is frequently employed in the screening of anticonvulsant agents that can raise the seizure threshold in the brain (White *et al*,. 1998). The mechanism of action of PTZ is by inhibition of GABA-A receptors and GABAergic neurotransmission. GABA is the major inhibitory neurotransmitter. Agents that enhance GABAergic transmission such as barbiturates and benzodiazepines are known to be protective against PTZ-induced seizure (Amabeoku *et al*., 2007). Activation of the N-methyl-D-aspartate (NMDA) receptor system and T-type Ca2+ currents are involved in the initiation and propagation of PTZ-induced seizures (Velisek *et al.*, 1999). The extract at the doses used produced a significant ($p < 0.05$) dose-dependent anticonvulsant activity against the PTZ-induced seizures as shown by increase in the onset and decrease in the duration of convulsions as compared to the control. This anticonvulsant effects shown by the extract against seizures induced by PTZ might possibly be due to potentiation of GABA-mediated inhibition in the brain, inhibition of T-type Ca2+ currents or blockade of glutaminergic neurotransmission mediated by NMDA receptor. Also, the 80% protection provided by the extract at 400 mg/kg body weight, compared to the 100% protection of the positive control, Sodium Valproate. The dose-dependent activity of the extract, therefore suggest better anticonvulsant activity at higher doses.

The extract protected 60% of the mice at doses of 100 and 200 mg/kg body weight, while 80% of the mice were protected at dose of 400 mg/kg (Fig. 3).

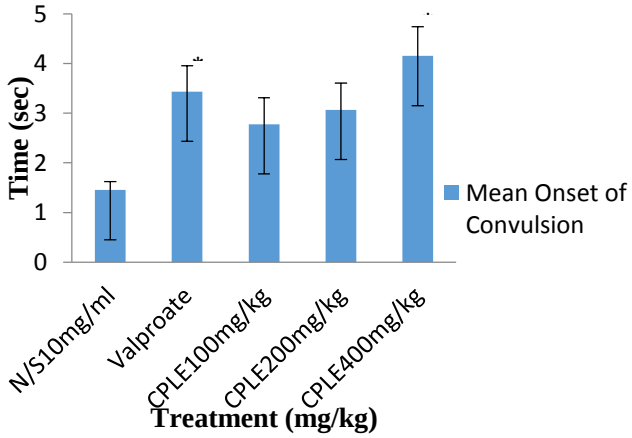

FIGURE 1: Effect of Treatments on Onset of Pentylenetetrazole-induced Convulsions in mice
N/S=0.9% Normal Saline (10 ml/kg), CPLE= *Carica papaya* Leave Extract (100, 200, 400 mg/kg body weight), Valproate 200mg/kg, n=5.

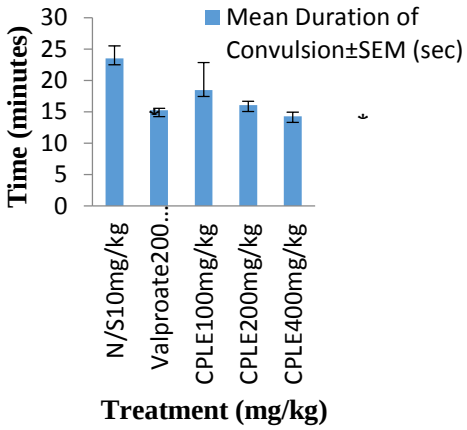

FIGURE 2: Effect of Treatments on Duration of Pentylenetetrazole-induced Convulsions in mice N/S=0.9% Normal Saline (10 ml/kg), CPLE= *Carica papaya* Leave Extract (100, 200, 400 mg/kg body weight), Sodium Valproate 200mg/kg, n=5.

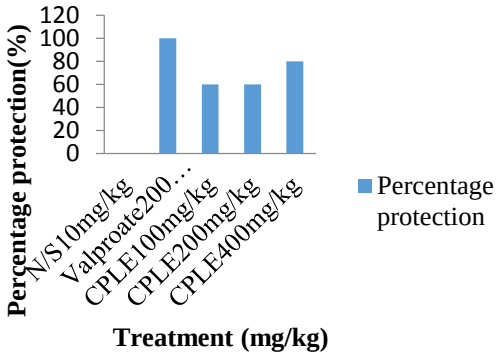

FIGURE 3: Effect of Treatments on Percentage protection (%) induced by Pentylenetetrazole in miceN/S=0.9% Normal Saline (10 ml/kg), CPLE= *Carica papaya* Leave Extract (100, 200, 400 mg/kg body weight), Sodium Valproate 200mg/kg, n=5.

In the MES-induced convulsions, the extract at doses of 100, 200 and 400mg/kg delayed the onset of seizure from 3.60±0.24 (normal saline) to 4.4±0.51, 5.4±0.24, and 8.4±0.24 respectively (Fig. 4). It also shortened the recovery time from 16.80±7.04 to 11.20±493, 10.40±4.00 and 4.00± 1.26 respectively (Fig. 5). However, these differences were not significant. The standard antiepileptic drug, Phenytoin (20 mg/kg) protected all the chicks used, delayed the onset of seizure from 3.6±0.24 to 6.4±3.97 and shortened the recovery time from16.80±7.04 to 2.60±1.60.

The maximal electroshock model is arguably the most useful tool in the identification of anticonvulsant compounds (Castel-Branco., *et al* 2009). The result obtained in this study, have demonstrated potential anticonvulsant property of *C papaya* leave extract. The extract at doses used produced a dose-dependent anticonvulsant activity against MES. At 200 mg/kg and 400 mg/kg body weight, it was able to provide100%protection to the chicks from HLTE, which compared favourably with the positive control. However, results showed that extract produced an insignificant ($p \leq 0.05$) increase in the onset and decrease in the duration of convulsion at the three doses administered. The MES model involved inactivation of sodium ion channel and this could possibility imply that the anticonvulsant mechanism of extract does not involve prolongation of sodium ion inactivation. Agents that prolong HLTE due to MES are said to be useful in the management of generalized tonic-clonic and partial seizures (Browning 1985; Maiha, 2009). The ability of the extract to provide 100% protection to the chicks against MES-induced seizure is thus an indication of potential anticonvulsant activity.

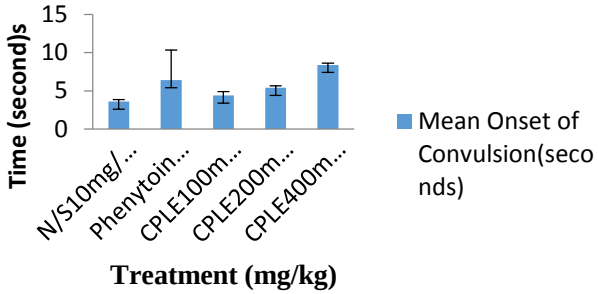

FIGURE 4: Effect of Treatments on Onset of Maximal Electroshock-induced Convulsions in chicks N/S=0.9% Normal Saline (10 ml/kg), CPLE= *Carica papaya* Leave Extract (100, 200, 400 mg/kg body weight), Phenytoin 20mg/kg, n=5.

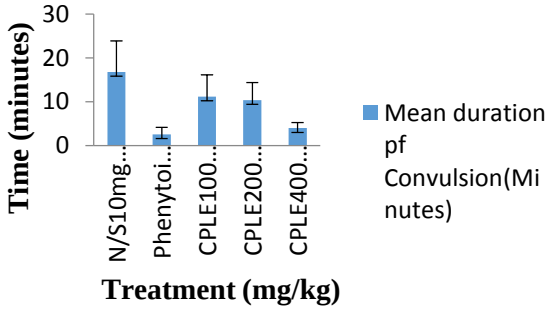

FIGURE: 5 Effect of Treatments on Mean Duration of Maximal Electroshock-induced convulsions in chicks N/S=0.9% Normal Saline (10 ml/kg), CPLE= *Carica papaya* Leave Extract (100, 200, 400 mg/kg body weight), Phenytoin 20mg/kg, n=5.

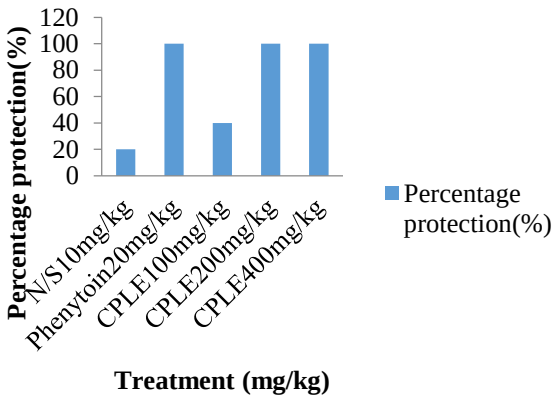

FIGURE: 6 Effect of Treatments on Percentage protection (%) induced by Maximal electroshock in chicks
N/S=0.9% Normal Saline (10 ml/kg), CPLE= *Carica papaya* Leave Extract (100, 200, 400 mg/kg), Phenytoin 20mg/kg, n=5

The extract at dose of 100 mg/kg body weight protected 40% of the chicks against maximal electroshock convulsion, while at doses of 200 and 400 mg/kg it provided 100% protection (Fig. 6).

Conclusion
Findings obtained from present study showed that *C papaya* leaves extract possesses dose-dependent anticonvulsant activity in mice and chicks. Although activities of extract were lower when compared to the standard drug Phenytoin and Sodium Valproate, its safety, availability, affordable and anticonvulsant activities suggests its potential for use in the management of convulsions especially in our rural areas.

Conflict of interest
The authors declare no conflict of interest.

Authors' Declaration
The authors hereby declare that the work presented in this articles is original and that any claims relating to the content of this article will be borne by them.

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