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Journal Name:	Journal of Pharmaceutical Research International
Manuscript Number:	Ms_JPRI_42430
Title of the Manuscript:	Mechanism of Anticonvulsant Effects of Ethanol Leaf Extract and Fractions of Milicia Excelsa (Moraceae) in Mice.
Type of the Article	Original Research Article

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	<p>The authors investigated anticonvulsant effects of various fractions of <i>Milicia excelsa</i> (welw.) C.C. Berg using 3 models of epilepsy.</p> <p>There are several concerns particularly with regards to methods and results</p> <p>Introduction: Authors have not provided sufficient evidence to support their hypothesis that <i>Milicia excelsa</i> (welw.) C.C. Berg produces anticonvulsant effects. Evidence to indicate the possible anticonvulsant efficacy of this plant should be included. Are there any studies on isolated chemicals from this plant with pharmacological effects that indicate possible anticonvulsant activity?</p> <p>The objectives of this study are not clear. It seems that the 1st part of experiments was designed to screen various fractions for antiepileptic activity and the 2nd to investigate the mechanisms of action. However, all three chemoconvulsants used in this study have well established mechanisms of action. Therefore if the test drug shows efficacy against a particular chemoconvulsant-induced epilepsy the mechanism can be predicted without the 2nd part of the experiment.</p> <p>Methodology is very confusing. To test the drug in each of the models there are 5 groups and 3 of them have received test drug (EME) in 3 different doses. Rationale to choose these doses is not stated.</p> <p>None of the other fractions (HF, EAF, BF, and AF) were tested under methods 2.5.1, 2.5.2 and 2.5.3, although the objective stated on page 3, lines 44 and 45 is ".....to investigate the anticonvulsant potential of the ethanol leaf extract, 45 HF, EAF, BF, and AF using mice models."</p> <p>Study design described to investigate mechanisms of action is extremely poor. Why was AF used instead of other fractions? Line 106 states use of "most active fraction (AF)". How authors reached that conclusion??</p> <p>Authors have investigated 3 mechanisms including GABA antagonism, 5-HT antagonism and NOS inhibition. However, it is not clear what prompted authors to explore only these 3 mechanisms. And the mechanisms were investigated only in PTX model, the rationale for which is not stated.</p>	<p>One of the earlier reported isolated compounds from the leaf of <i>Milicia Excelsa</i> is ursolic acid which is found in many other medicinal plants and has been shown to possess anticonvulsant effects. Therefore, ursolic acid, either in additive or synergy with other phytochemicals in <i>Milicia excelsa</i> leaf could be responsible for the anticonvulsant effect of the <i>Milicia excelsa</i> leaf. Line 41-43 and Line 256-261.</p> <p>All the three chemoconvulsants used in this study have well established mechanisms of action but further involvement of serotonergic and nitric oxide signalling pathways in the anticonvulsant effect of AF were investigated in the 2nd part. Involvement of GABA using Flumazenil was also carried out to corroborate the established mechanism of the first part.</p> <p>Based on the outcome of the preliminary acute toxicity study (line 40-43), the doses for the neurobehavioural activities were conducted at 1/20, 1/10 and 1/5th of the LD50 (5000 mg/kg) that corresponded to 250 (low), 500 (medium) and 1000 mg/kg (high) were selected as seen from other works in literature [line 204-206].</p> <p>Correction effected line 85-86; line 95-96 and line 105-107 of the main manuscript.</p> <p>AF was used and considered as the most active fraction because it gave the highest percentage protection of 83.3 and 100 at the highest dose of 1000 mg/kg in PTZ-, and PTX-induced convulsion models [line 112-114].</p> <p>Earlier reports have implicated GABA antagonism, 5-HT antagonism and NOS inhibition in anticonvulsant effects of medicinal plants. We explored these mechanism to suggest if AF was acting via any of these mechanism and to be able to suggest also if there exist any probable functional interaction between 5-HT, NO and GABA in the anticonvulsant effect of AF as suggested in other previous studies [Line 228-235].</p> <p>Since AF produced consistent anticonvulsant effects in PTX-, and PTZ-induced convulsion models, and these chemoconvulsants act via GABA receptor neurotransmission, the mechanism of anticonvulsant effect of AF was therefore investigated in PTX-induced convulsion model [Line 231-233].</p> <p>Result of L-NNA + diazepam is included. Table 4</p> <p>The methods have been corrected to include HF and BF in the methods as earlier indicated above.</p> <p>HF and BF were included in results as pointed out in 3.1 Effects of HF, EME,</p>



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	<p>No control groups with only inhibitors without extract? It appears from table 4, such groups were probably included. If this is true, L-NNA + diazepam group is missing Number of animals per group?</p> <p>Results are not at all in line with methods. For example:</p> <p>3.1 Effects of HF, EME, EAF, BF and AF of Milicia excelsa on.... But the table does not show the effects of HF and method describes only use of EME. Same is true for the results of other models. Methods have stated 5 groups of animals in 2.5.1, 2.5.2 and 2.5.3 but the corresponding results in tables 1, 2 and 3 show 11 groups.</p> <p>Table 1, column 4 row 11 shows a value of 1587.5 ± 2125. SEM is bigger than mean. Is it 2125 or 212.5?</p> <p>Table 3 shows effect of pentobarbitone but corresponding method descriptions states use of diazepam. Diazepam is stated as “DZP” at places and “DPZ” at other places.</p> <p>Line 223: “Since AF produced consistent anticonvulsant effects in all the convulsion models used” is wrongly stated. No protective effect of AF was seen in SCN model (table 3).</p> <p>Line 251-2512 state that the magnitude of activity of the fractions was of the order AF > EAF > HF > BF. Nowhere in the manuscript effects of HF and BF are presented. So how authors reach this conclusion?</p> <p>Authors propose that AF acts via three mechanisms that were investigated. However, it is hard to understand how aqueous fraction which is expected to contain all water soluble constituents manages to cross the blood brain barrier and exert the said effects. Authors should provide the explanation for the same.</p> <p>The discussion is largely based on the evidence for similar activities of other plants. Authors should rather focus on the extracts investigated, their possible constituents and targeted mechanisms.</p>	<p>EAF, BF and AFHowever, HF and BF were only discussed under results but excluded from the table because no statistical significance were shown by them on all the parameters of onset of clonic, tonic convulsion and death latencies.</p> <p>Error corrected and highlighted in table 1. It is 1587.5 ± 212.5</p> <p>Error corrected and highlighted on table 4</p> <p>The statement has been changed to “Since AF produced anticonvulsant effects in PTZ-, and PTX-induced convulsion” Line 228</p> <p>HF and BF have been excluded since the effects of HF and BF were not presented anywhere in the manuscript as rightly pointed out by the reviewer Line 270-271. So also HF and BF have been deleted from the abstract.</p> <p>How the AF transversed the blood brain barrier (BBB) to exert the observed anticonvulsant effect could not be established in this study. It can probably be suggested that the phytocompounds in AF could transverse the BBB by active transport since hydrophilic drugs are substrates for drug transporters of the BBB. Moreso, previous studies have demonstrated the anticonvulsant effects of AF of medicinal plants. Line 262-266.</p> <p>The manuscript has been edited to de-emphasise the discussion on the evidence for similar activities of other plants. Line 212-214, 220-221 and 225-227.</p>
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<u>Minor</u> REVISION comments	There are several grammatical errors that need correction. Some of the results could have been better presented in graphs	Efforts have been made to correct any grammatical errors. Tables were preferred to graphs because tables could contain more information than graphs without being clumsy. For example more graphs will be needed to present the onset of clonic, tonic convulsions and death latency for each chemoconvulsant without being clumsy whereas a table can clearly present all the information.
<u>Optional/General</u> comments	The manuscript is poorly written with several inconsistencies particularly with regards to methods and results. The conclusion which states high efficacy of aqueous fraction is hard to understand because aqueous compounds do not easily cross the blood brain barrier.	The inconsistencies regarding the methods and results have been reconciled. Addressed as above