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PART 1:

Journal Name:	**British Journal of Pharmaceutical Research**
Manuscript Number:	**2013_BJPR_4833**
Title of the Manuscript:	**Anticholinesterase, Antioxidant and Nitric Oxide Scavenging Activity of the Aqueous Extract of Some Medicinal Plants**
Type of the Article	**Research paper**

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- This form has total 7 parts. Kindly note that you should use all the parts of this review form.

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PART 2: 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		
Minor REVISION comments	13 Neurodegenerative disorders such as Alzheimer's and Parkinson's diseases are 18 factor in the pathogenesis of neurodegenerative disorders like Alzheimer's disease [2]. 19 an integral process in the cellular metabolism and ~~during~~ oxidation, free radicals ~~are~~ 20 produced ~~that~~ have unpaired electron [3-6]. 21 electron are highly reactive and produce cellular injury~~damage~~ by causing membrane lipid 24 the drugs that can increase the cholinergic activity as well as are antioxidants. A variety of 25 anticholinesterases and antioxidants have been used, however, search for more effective 26 and safer agents continues. (references?) 27 Several agents original from plants ~~from plant origin~~ have previously been investigated for their	

	AchE inhibitory 28 and antioxidant properties. In the present study we evaluated the AchE inhibitory, total 29 antioxidant and NO scavenging activity of the aqueous extracts of the seeds of *Ocimum* *30 basilicum* (OB), rhizomes of *Curcuma longa* (CL) and berries of *Solanum nigrum* (SN). 58 were ~~was~~ estimated for all three extracts and their ~~combination by a~~ linear regression analysis 59 between the percentage inhibition and ~~versus~~ the extract concentrations was performed by using 63 Acetylcholine iodide is used as the substrate. When acted upon by the ~~enzyme~~ AChE it 64 breaks down to thiocholine and acetate. 63 When acted upon by the ~~enzyme~~ AChE, acetylcholine iodide, the substrate, ~~it~~ 64 was broken ~~breaks~~ down to thiocholine and acetate. 66 colour. The changes in the intensity of yellow color over a period of time, which can be estimated using a 67 UV spectrophotometer, were calculated and represented ~~is~~ ~~a measure of~~ the activity of AChE. 72 with pH 7.4. Physostigmine 1mM was prepared	

	in distilled water and was used as a reference 73 standard. The aqueous plant extracts were dissolved in distilled water so as to get the 74 desired concentrations. 80 blank. Solutions were again incubated for ? min. with intermittent shaking. 85 **2.2.4 Calculation of ~~e~~Enzyme ~~a~~Activity** 87 averaged within each three min run. The rate of reaction was calculated according to 88 the following formula: 96 100%. By comparing with blank, percent enzyme activity and percent inhibition of enzyme 97 activity of the extracts were ~~was~~ calculated. 99 **2.3 Evaluation of ~~a~~Antioxidant ~~a~~Activity** 101 *al.*, in 1999 [15]. Each extract was used at ~~in~~ the concentration equivalent to IC50 for AchE 102 inhibition. 155 The method was first validated and the absorbances were ~~was~~ measured in the absence or presence of different concentrations of inhibitor, physostigmine, at 0.5 – 1.5 mmol ~~of as~~ 156 ~~well as in the presence of different~~	

~~concentrations of physostigmine (0.5 – 1.5 mmol).~~

Questions

1. Stock physostigmine 1 mM (line 72) so 0.5 – 1.5 mmol = 0.5 – 1.5 mM ? (∵ mM = mmol/L)

If so, it should be changed stock concentration of physostigmine?

2. Were 0.5 – 1.5 mM final concentrations (in 3.3 ml) ? because 50 µl of 1 mM physostigmine was added to the final total volume 3.3 ml.
3. Are the authors sure whether these concentrations are final concentration not added concentrations?

161 3.38 ± 0.05 and 3.88 ± 0.11 g~~m~~/l for SN, CL and OB, respectively.

165 The phosphomolybdenum assay was performed using ascorbic acid in the concentration
166 range of 1 to 5 mmol = 1 – 5 mM ? because concentration usually represents mole per volume (L or ml or µl) and figure 1 was labeled per l, mmol of ascorbic acid/l
167 estimated and expressed as equivalents of mM~~mol~~ of ascorbic acid.

169 mM~~mol~~ of ascorbic acid followed by SN and OB with a mean value of 2.12 ±0.11 and 1.88 ±

	170 0.18 mM~~mol~~ of ascorbic acid respectively. 172 consisting of OB, CL and SN was equivalent to 5.95 ± 0.32 mM~~mol~~ of ascorbic acid. The 173 antioxidant capacity of herbal combination was significantly higher than all of each ~~its constituent~~ 174 extract~~s~~ (P<0.05 vs CL and P<0.001 vs OB or ~~and~~ SN) (Fig 2). 187 The NO scavenging activity of three extracts and their combinations were ~~was~~ estimated in a 188 diazotization reaction. The calibration curve for sodium nitrite (10-70 mmol/l) was used to 189 calculate the NO scavenging activity of test drugs which was expressed as equivalent to 190 mM~~mol~~ of sodium nitrite (Fig 3 ⇒ Concentration of Sod nitrite (mmol/l)). 191 maximum NO scavenging activity, which was equivalent to 29.78 ± 1.28 mM~~mol~~ of sodium 192 nitrite. The NO scavenging activity of SN and OB were ~~was~~ equivalent to 11.71 ± 1.84 and 11.34 193 ± 2.30 mM~~mol~~ of sodium nitrite, respectively. The NO scavenging activity of CL was 194 significantly higher than those of two other extracts (P<0.01). The NO scavenging activity of the combination of three extracts was equivalent to 39.83 ± 195 1.82 mM~~mol~~ of sodium nitrite and this 196 was significantly higher than their constituent extracts (P<0.001vs OB or ~~&~~ SN, P<0.05 vs CL).	

	197 (Fig 4) On figure 4, Y-axis should be mM of sodium nitrite, instead of mmols of sodium nitrite Legend: Fig 4: Nitric oxide scavenging activity of three plant extracts and their combination 205 **P*<0.001 versus OB or ~~and~~ SN; #*P*<0.05 versus CL 208 Present study demonstrated AchE inhibitory, antioxidant and nitric oxide scavenging activity 213 options. Inhibition of AchE has been shown to enhance cholinergic transmission in the brain and 218 recognized as remarkable alternatives [22]. As the Ellman reaction measured~~s~~ both AchE and 219 BchE activity, the extracts evaluated in this study were found to have significant ~~a~~AchE and 220 BchE inhibitory activity. Oxidative stress as an underlying pathophysiological process is also 225 scavenging them or by reducing their production~~s~~. Nowadays, ~~Existing~~ anticholinesterase drugs used to treat Alzheimer's disease such as 226 tacrine, donepezil, galantamine and heptylphysostigmine cause several adverse effects such 227 as hepatotoxicity.	

	228 peripheral cholinergic adverse effects and ~~a~~ narrow therapeutic windows. Therefore, 229 investigations of ~~for~~ newer drugs that possess both AchE inhibitory and antioxidant properties 230 and are safe are ~~is~~ extremely important not only for treatment Alzheimer's disease but also for prevention of the neuronal cell damage from ROS. **Question:** results are very interesting, if different 2 combinations are also included. Have the authors tried two combinations such as CL+SN, CL+OB, SN+OB and compared the inhibitory activities (anticholinesterase, antioxidant and NO Scavenging Activity) to those of three combination? References 315 20. J. R. Hodges, "Alzheimer's centennial legacy: origins, landmarks and the current 316 status of knowledge concerning cognitive aspects," *Brain*~~, vol. 129, pp. 2811-2822, Nov. 2006~~ Brain. 2006;129:2811-2822.	

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Optional/General comments	This article is very good and clear. Authors should mention a process how to get plasma from normal subjects (at least sex and range of ages and) with inform consents through the ethical committee.	

Note: Anonymous Reviewer