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Full Length Research Paper

Influence of leaf extracts from *Melia azedarach* L. on butyrylcholinesterase activity in rat liver

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Four types of extracts were prepared from *Melia azedarach* L. (Meliaceae) leaves: Aqueous, potassium phosphate buffer (pH 7.2), hydroethanolic solution 70:30 and hydroethanolic solution 50:50. Different concentrations of these extracts were investigated for the effect on butyrylcholinesterase (BuChE, EC 3.1.1.8) activity in homogenates rat livers. The introduction of *M. azedarach* extracts in the reaction mixture produced a variety of inhibitions (> 45 to 100%), independent on its concentration (0.5 to 2.0 mg.ml^{-1}) and extract type. A clear explanation for this interaction between *M. azedarach* and active site of butyrylcholinesterase is still missing. The UV-VIS (200 to 400 nm) absorption spectrum and the phytochemicals tests of the extracts show diversity of compounds including flavonoids, a known inhibitor of butyrylcholinesterase. On the other hand, decreased butyrylcholinesterase activity in homogenates rat livers was not correlated with flavonoids content, suggesting that other compounds may have contributed to the inhibitory capacity of this plant.

Key words: Enzymatic activity, flavonoids, medicinal plants.

INTRODUCTION

Butyrylcholinesterase (BuChE; EC 3.1.1.8) is of pharmacological and toxicological importance, because it catalyzes cleavage ester-containing drugs. Many questions have been solved, but a number of others are still awaiting the necessary experimental evidence. The physiological role of butyrylcholinesterase is still unknown. Some studies suggest that butyrylcholinesterase plays a role in lipid and lipoprotein metabolism and serves as a co-regulator of cholinergic neurotransmission because it also efficiently hydrolyzes acetylcholine (Carmona et al., 2000; Lucić et al., 2002; Çokugras, 2003).

Several authors suggest that butyrylcholinesterase could be involved in the development of some forms of dementia, including Alzheimer's disease (Darvesh et al., 2003). Therefore, alterations in butyrylcholinesterase activity can affect drugs metabolism, lipid metabolism and acetylcholine cleavage, resulting in different clinical symptoms. Herbs have been used in nutrition, as fragrances, pesticides, medicines and other purposes. It has been shown that the leaves, barks and other parts of *Melia azedarach* L. (Meliaceae) have insecticidal and several pharmaceutical properties. This tree is known as chinaberry; is a deciduous tree native to China, India and Persia, but it is widely distributed all over the world, especially in the tropics, because of its climatic tolerance (Alche et al., 2002).

Concoction and extracts of *M. azedarach* have been used orally and topically by the traditional medicine of many countries as an antiviral agent, diuretic, febrifuge and used against intestinal disorders (Senthil-Nathan et al., 2005; Samudram et al., 2008). A number of flavonoids, limmonoids, tannis, sterols, saponines and triterpenoids have been detected in that plant (Simmonds et al., 1992; Jorge et al., 2009; Nyunja et al., 2009). In many species, including man, these compounds act on important reactions metabolic (Chaturvedi and Segale, 2007; Phua et al., 2008; Chiffelle et al., 2009). Chaturvedi and Segale (2007) demonstrated that *M. azedarach*

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extracts are able to decrease glucose levels on induced hyperglycemia in rats by a mechanism unclear. Its hepatoprotective activity is documented and it results in regeneration of hepatocytes. Samudram et al. (2008) interpreted this action as an inhibition of free radicals generation. There is also a report about an antiulcer activity.

In a study aiming to elucidate the mechanism of this action, it was concluded that plant is involved in synthesis of cytoprotector glycoproteins (Hanifa and Al-Khatib, 1984). Although it remains unclear which of the compounds are the active ones; flavonoids and phenolic compounds have received attention because of new findings regarding their biological effects. A large number of mechanisms of action have been attributed to these compounds, including free radical scavenging activity, lipid peroxidation inhibition and butyrylcholinesterase inhibition (Gao et al., 2000; Djeridane et al., 2006; Katalinić et al., 2010).

Plants extracts have been subject of a lot of research in order to obtain compounds able to inhibit butyrylcholinesterase. In addition, when examining the available data, one of the questions that can be raised concerns the consequences of this plant for the butyrylcholinesterase activity. However, no responses about this question have been found in literature and this is precisely the gap that the present work intends to fill.

As an extension of previous work performed in our laboratory, the present investigation was planned for measuring the action of *M. azedarach* on butyrylcholinesterase activity in the rat liver. Different types of extracts (aqueous, potassium phosphate buffer and hydroethanolic solution) were used to visualize butyrylcholinesterase-*M. azedarach* interactions. The results should contribute to a better understanding of the pharmacologic and toxic effects of *M. azedarach* on biological system.

MATERIALS AND METHODS

Chemicals

Propionylthiocoline iodide (PTCh) and 5'5`-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Sigma Chemical Co., USA. All other chemicals were from the best available grade (98 to 99.8% purity).

Plant material

Fresh green leaves of *M. azedarach* were collected in the month of July 2010. The specimens harvested from trees grown in Cascavel city (Brazil) were identified at the Laboratory of Botany of the State University of Western Parana, Brazil. The plants were identified by biologist Dr. Norma Catarina Bueno. The leaves were collected, shade-dried and crushed to powder for preparation of aqueous, potassium phosphate buffer and hydroethanolic solution (70:30) extracts. The hydroethanolic (50:50) extract was prepared with fresh chopped leaves.

Extracts preparation

All extracts were prepared for a final concentration of 30% (m/v). The amount of starting material (leaf powder) was always 60 g. For preparing the aqueous extract, the leaf powder was boiled in 200 ml of distilled water. Boiling was stopped when the water volume was reduced to ¼ of the initial volume (0.635 g) and then lyophilized (0.512 g) (Chaturvedi and Segale, 2007). Extraction with 10 mM potassium phosphate buffer (pH 7.2) containing 350 mM KCl was done using a commercial blender. The sap obtained was filtered and centrifuged at 5.000 g for 1 h. Pellets were discarded and the supernatants lyophilized (1.480 g) (Alche et al., 2002).

When using the hydroethanolic solution 70:30 (v/v) the powder was extracted for 3 h and filtered. This operation was repeated three times. The filtrate was freed from solvent by evaporating it in a rotary evaporator under reduced pressure. The final residue collected (5.750 g) was a thick paste (Yatsuda et al., 2005).

For preparing the hydroethanolic solution 50:50 (v/v) extract, freshly chopped leaves were mixed with the solvent at room temperature. After 24 h the mixture was filtered and the final residue collected (7.660 g) (Jorge et al., 2009).

Characterization of the extracts

The presence of the following compounds was investigated by means of standard qualitative procedures: alkaloids, steroids, tannis, flavonoids, saponins and coumarins. The presence of alkaloids was searched by means of the Dragendorff's and Mayer's reagent (Wall et al., 1954). The Liebermann-Burchard reaction was used in order to investigate the presence of steroids (Nath et al., 1946). Tannins were searched by means of the chemical reaction with iron salts (Michelin et al., 2005). The Shinoda's reaction was employed in the analysis of flavonoids (Sahu-Vinod et al., 2010). The tests for saponines and coumarins were those described by Barbosa-Filho et al. (2005). Absorption spectra of the extracts were recorded using methanolic 250 $mg.L^{-1}$ solutions in the wavelength range from 200 to 400 nm with a resolution 1 nm.

The batochromic shift was evaluated after treatment of the extracts with aluminum chloride and hydrochloric acid at the maximum absorption wavelength and the UV spectrophotometry by comparing the obtained data with those in literature. The UV spectra were obtained on a FEMTO-800 XI spectrophotometer.

Quantification of residual alcohol and estimation of flavonoids content

The residual alcohol content of the hydroethanolic extracts was measured according to Widmark (1964). Essentially, residual alcohol was oxidized by means of dichromate, and the excess of dichromate used in the oxidation was determined iodometrically. The results were expressed as mg alcohol per g extract. Concentration of flavonoids of all extracts was determined spectrophotometrically using a method based on the formation of a flavonoid-aluminium complex. The sample was mixed with a methanolic aluminum chloride solution.

After incubation at room temperature, the absorbance of the reaction mixture was measured at 430 nm (Quettier-Deleu et al., 2000). The calibration curve was obtained with quercetin. The absorbance was measured in a FEMTO 600S spectrometer and the flavonoids contents were expressed as mg per g of quercetin equivalents (QE).

Animal treatment and homogenate preparation

Homogenates were prepared from rat liver by adaptation of the

Table 1. Phytochemical composition of the *Melia azedarach* L. extracts.

Extract	Compound
Aqueous	Flavonoids and saponins
Potassium phosphate buffer (pH 7.2)	Coumarins, flavonoids, saponins and steroids
Hydroethanolic 70:30	Alkaloids, coumarins, flavonoids, saponins, steroids and tannis
Hydroethanolic 50:50	Alkaloids, flavonoids, saponins, steroids and tannis

Characterization of extracts was assayed as described in "Material and Methods" section.

methodology described by Cimasoni (1966). Male albino rats (Wistar), weighing 180 to 220 g, were fed *ad libidum* with a standard laboratory diet (Nuvilab®). For the removal of the liver, animals were decapitated and exsanguinated. Their livers were removed immediately and cut into small pieces. These fragments were suspended in phosphate buffer (pH 7.2).

Homogenization was carried out in the same medium by means of glass homogenizers on ice. Homogenization was followed by differential centrifugation at 536 g for 10 min and 4.000 g for 10 min. Their supernatant fraction was used immediately for biochemical assay. Protein content of the homogenate was measured using the Folin-phenol reagent and bovine-serum albumin as a standard (Lowry et al., 1951). Animal management was conducted according to the Brazilian regulations for the use of laboratory animals and the ethical principles for animal management.

Measurements of butyrylcholinesterase activities

The butyrylcholinesterase activity in the liver was assayed by method of Ellman et al. (1961), using propionylthiocoline as substrate. The assay contained 100 mM phosphate buffer (pH 8.0), 0.25 mg protein of the liver supernatant fraction and 0.5 mM DTNB. After determination of the blank, the reaction was started by addition of 5 mM propionylthiocoline iodide. The extracts were added to the mixture just before starting the reaction. The change in extinction was recorded at 405 nm for 120 s. The specific enzyme activities were calculated as nmol of substrate hydrolysed.min^{-1}. mg $protein^{-1}$. The influence of *M. azedarach* on butyrylcholinesterase activity was measured in incubations containing extracts with final concentrations between 0.5 and 2 $mg.ml^{-1}$. Appropriate control experiments were run in order to exclude alcohol residue effects.

Treatment of data

Data are expressed as mean ± standard errors. Statistical analysis was performed using Student's t-test and ANOVA. Differences were considered significant at $P \leq 0.05$. Correlations were calculated using the Prism® package.

RESULTS AND DISCUSSION

Characterization of the extracts

To identify compounds present within the *M. azedarach* extracts, phytochemical analysis and absorption spectra were employed. The qualitative phytochemical screening revealed several classes of compounds, confirming the results of the other authors (Jorge et al., 2009). In all extracts, two compounds, saponins and flavonoids predominated (Table 1). For all extracts, the spectra showed absorption maximum in the ultra-violet range with a peak between 255 and 260 nm. However, the hydroethanolic extracts also showed peak or shoulder at proximal VIS region (between 343 and 350 nm) (Figure 1).

UV data with characteristic batochromic showed the presence of hydroxyl groups. The aluminum form stable complexes with the C4 keto group and either the C3 or C5 hydroxyl group of flavones and flavonols and forms labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids (Figure 2). These complexes have shown the absorption maximum at longer wavelength. UV λ_{max}^{MeOH} nm: aqueous extract: 256.1; (+$AlCl_3$): 269.7; (+$AlCl_3$ + HCl): 263.1; potassium phosphate buffer pH 7,2 extract: 255.7; (+$AlCl_3$): 270.0; (+ $AlCl_3$ + HCl): 263.3; hydroethanolic 70:30 extract: 259.1; 349.8; (+ $AlCl_3$): 271.1; 414.6; (+ $AlCl_3$ + HCl): 272.5; 357.7; 389.0; hydroethanolic 50:50 extract: 255.0; 343.5; (+ $AlCl_3$): 273.3; 410.0; (+ $AlCl_3$ + HCl): 275.1; 357.0; 388.2. The interpretation of spectrum data was comparable to the literature range, suggesting the flavonoids presence (Vieira et al., 2005; Lopes et al., 2007).

Estimation of flavonoids content

According to the UV-VIS absorption spectra and phytochemicals tests, flavonoids contents of extracts were determined. The amount of flavonoids varied in different type extract and ranged from 5.50 to 15.91 mg quercetin equivalent/g extract. The lowest levels were detected in aqueous and potassium phosphate buffer extracts when compared to the values observed in hydroethanolic extracts, are shown in Table 2.

Influence of alcohol on butyrylcholinesterase activity

Before carrying out the experiments, it was checked that the alcohol alone, in order to reach a final concentration in the range between 0.132 and 1.320 $\mu g.ml^{-1}$, used to make up hydroethanolic extracts, had influence on butyrylcholinesterase. As shown in Figure 3, alcohol

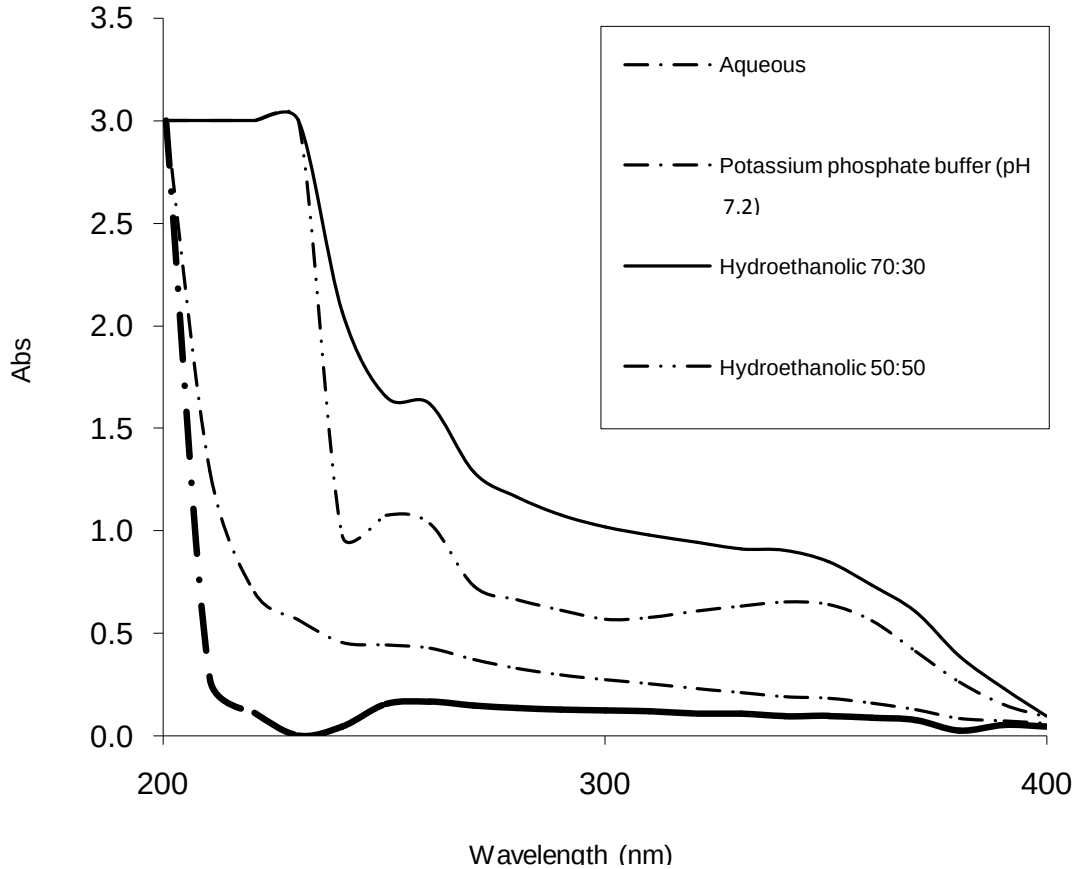

Figure 1. Absorption spectrum of the *Melia azedarach* L. extracts (250 mg.L^{-1}).

produced no effect on butyrylcholinesterase activity.

Butyrylcholinesterase activity

The results of effect of *M. azedarach* extracts on butyrylcholinesterase activity are illustrated in Figure 4. All four extracts prepared (aqueous, potassium phosphate buffer (pH 7.2), hydroethanolic solution 70:30, hydroethanolic solution 50:50) inhibited rat liver butyrylcholinesterase. The introduction of *M. azedarach* extracts in the reaction mixture produced a variety of inhibitions, independent on its concentration and extract type. However, inhibition potency of extracts do not presented considerable difference between different preparations ($P > 0.05$).

The lowest concentration (0.5 mg.ml^{-1}) of *M. azedarach* extracts induced a considerable inhibition of butyrylcholinesterase activity (> 45%), while concentrations beyond 1.0 mg.ml^{-1} progressively inhibited the activity of this enzyme. Full effect (~100% inhibition) was obtained for 1.0 mg.ml^{-1} (hydroethanolic 70:30 extract) and 2.0 (potassium phosphate buffer extract), that is, for a ratio of extract (mg)/protein (mg) of 4 and 7, respectively.

Inhibition potency of the *M. azedarach* extracts increased in the following order: potassium phosphate buffer (0.5 mg.ml^{-1}) < aqueous (0.5 mg.ml^{-1}) < aqueous (1.0 mg.ml^{-1}) < hydroethanolic 70:30 (0.5 mg.ml^{-1}) < aqueous (2.0 mg.ml^{-1}) < hydroethanolic 50:50 (0.5 mg.ml^{-1}) < hydroethanolic 50:50 (1.0 mg.ml^{-1}) < hydroethanolic 50:50 (2.0 mg.ml^{-1}) < potassium phosphate buffer (1.0 mg.ml^{-1}) < hydroethanolic 70:30 (2.0 mg.ml^{-1}) < potassium phosphate buffer (2.0 mg.ml^{-1}) < hydroethanolic 70:30 (1.0 mg.ml^{-1}) (Table 3).

These results show evidence for an interaction of *M. azedarach* with the active site of butyrylcholinesterase rat liver, but that relationship is unclear. Similar observations have already been reported in studies concerning other plants (Menichini et al., 2009; Kim et al., 2011). The decrease of activity enzymatic could be the consequence of the presence of different components in the plant tissues, in special flavonoids as demonstrated by Katalinić et al. (2010) and Senol et al. (2010).

The inhibition butyrylcholinesterase activity is one characteristic effect these compounds. If this is correct, hydroethanolic extracts should have an effective enzymatic inhibition. This observation was perfectly consistent with the fact that hydroethanolic extracts show a highest inhibitory effect on butyrylcholinesterase. On

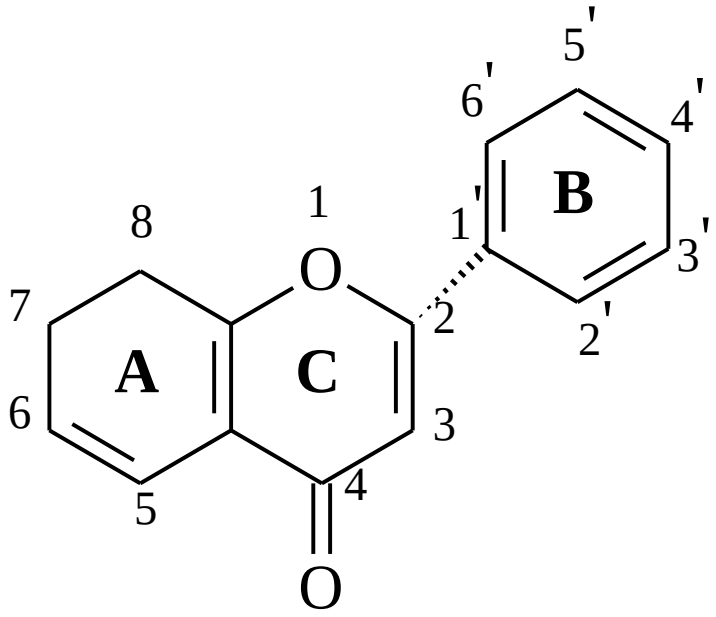

Figure 2. Basic structure of flavonoids. The potential presence of hydroxyl groups in C3, C4 or C5.

Table 2. Flavonoids contents for *Melia azedarach* L. extracts.

Extract	Flavonoids (mg QE/g dw)[a]	Flavonoids (mg QE/extract)[b]
Aqueous	5.50 ± 0.09	2.816
Potassium phosphate buffer (pH 7.2)	7.56 ± 0.23	11.19
Hydroethanolic 70:30	15.91 ± 0.10	91.48
Hydroethanolic 50:50	11.93 ± 0.42	91.38

Flavonoids contents of extracts were assayed as described in "Material and Methods" section. [a]Values are means ± S.E.M. of three separate determination. Data expressed as per gram of dry weight. [b]Represent flavonoids contents in each extract produced.

Table 3. Inhibitory effect of *Melia azedarach* L. extracts on butyrylcholinesterase of homogenates rat livers.

Extract	Extract (mg.ml^{-1})		
	0.5	1.0	2.0
Aqueous	56.3	58.9	78.9
Potassium phosphate buffer (pH 7.2)	45.8	90.5	100
Hydroethanolic 70:30	76.3	100	94.7
Hydroethanolic 50:50	78.9	87.9	87.9

Enzyme-extract inhibition was determined from at four experiments. Values are percent of control.

the other hand, the data analysis showed that there was a poor correlation between the flavonoids contents and butyrylcholinesterase activity. As shown in Figure 5, the correlation coefficient increased from hydroethanolic solution 50:50 < hydroethanolic solution 70:30 < aqueous < potassium phosphate buffer (pH 7.2). This causes poor correlation, however, cannot be inferred from the available data. Several possibilities can be considered. First, the flavonoids content does not incorporate all the inhibitory activity. It is known that flavonoids interact with many proteins in the organism (Van den Berg et al., 2000; Gutzeit et al., 2005).

Moreover, affinity for binding to butyrylcholinesterase active site is complex and dependent of the structure. In

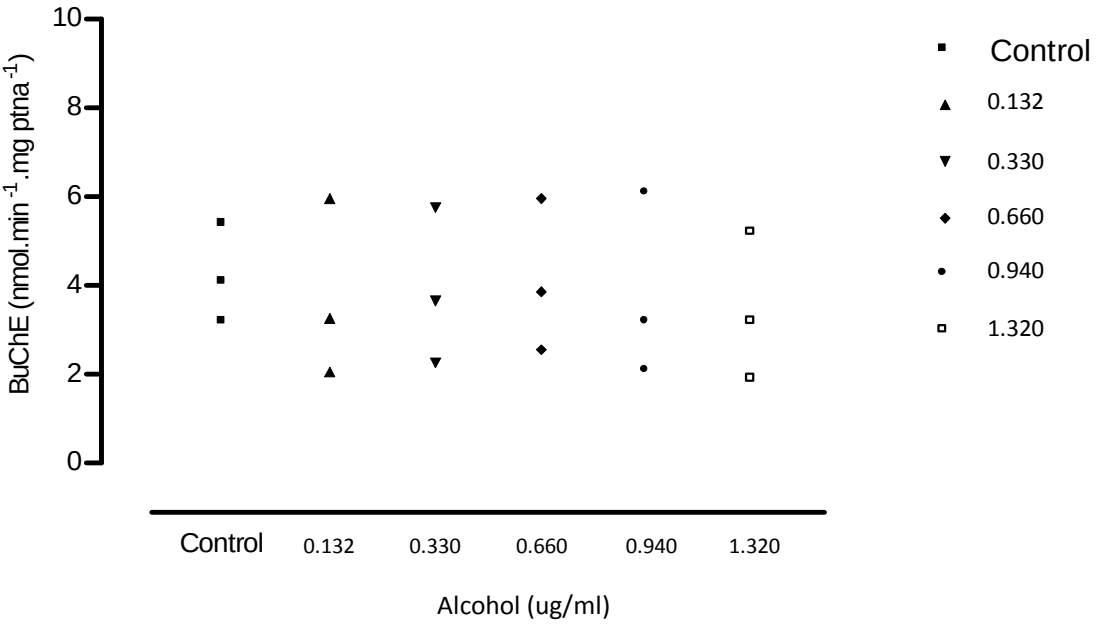

Figure 3. Activity butyrylcholinesterase in homogenates rat livers and influence of alcohol (expressed as nmol.min^{-1}.mg protein^{-1}). Homogenates were prepared and assayed as described in Material and Methods. Data represent the mean ± S.E.M obtained with 3 homogenates preparations.

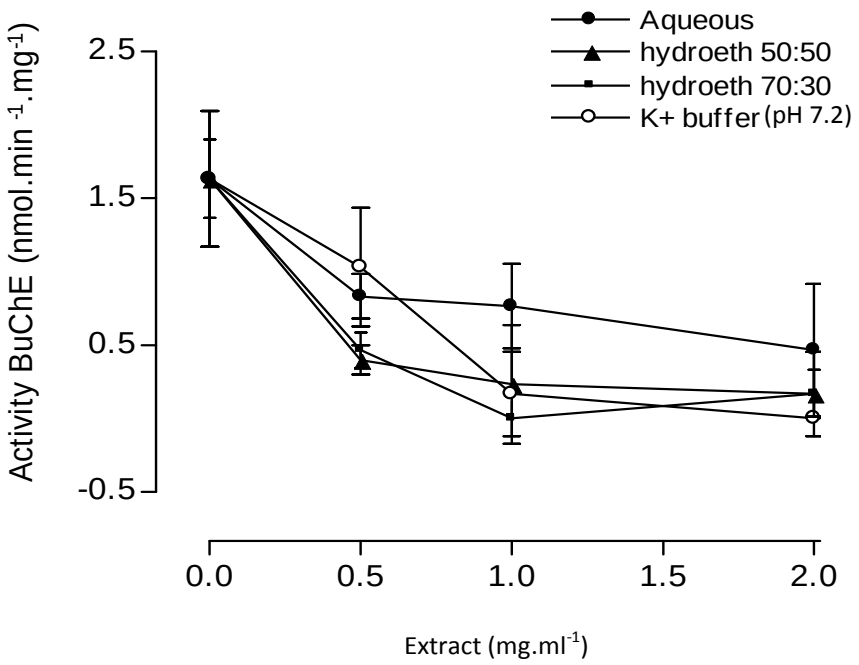

Figure 4. Innhibitory effect of extracts on butyrylcholinesterase of homogenates rat livers (expressed as nmol.min^{-1}.mg protein^{-1}). Homogenates were prepared and assayed as described in Material and Methods. Data are the means ± S.E.M obtained with 3 to 6 homogenates preparations.

addition, a study reveals that decreased activity depends on the number and the position of hydroxyl groups on the phenyl ring (Menichini et al., 2009). However, the presence of different flavonoids in extracts is relatively

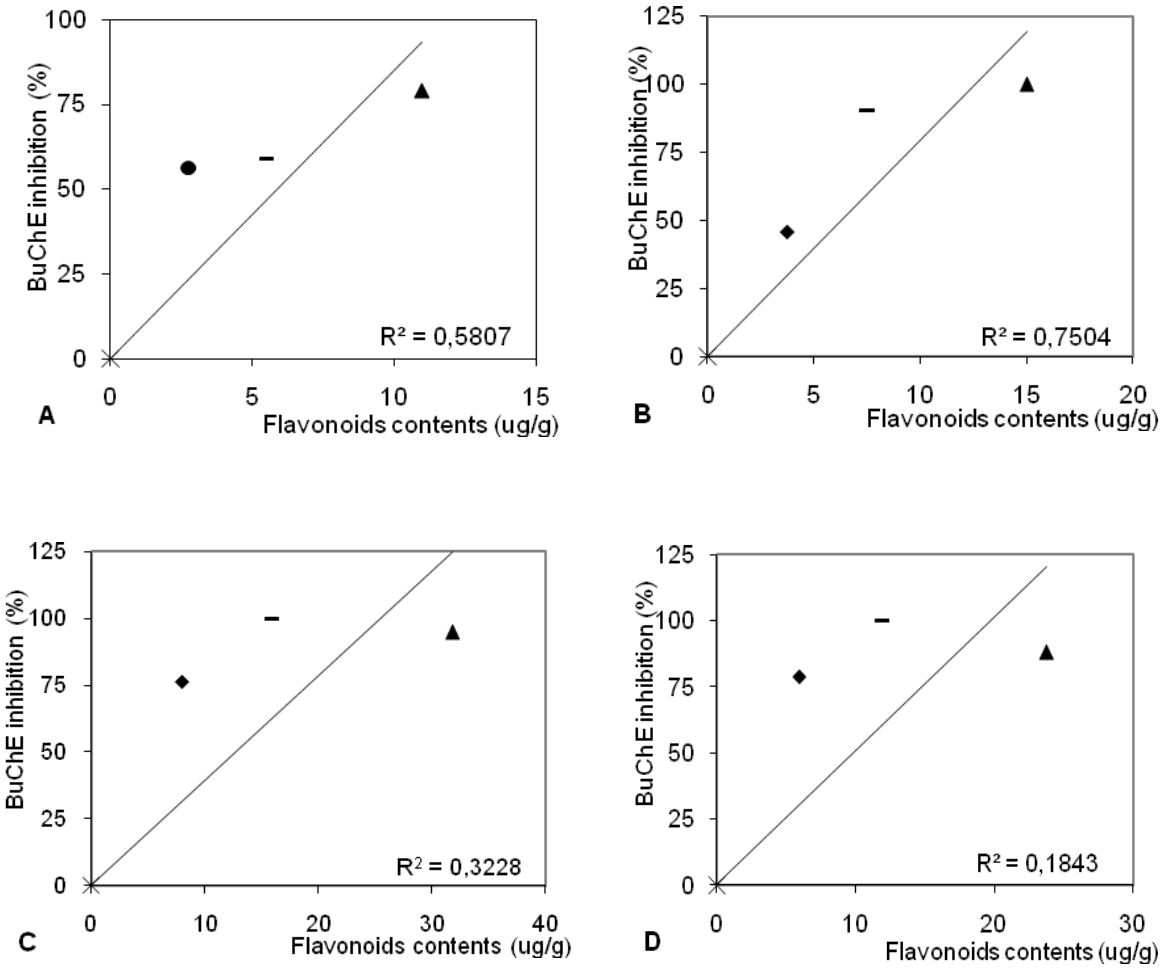

Figure 5. Linear correlation of inhibition butyrylcholinesterase activity with respect to the flavonoid content of *Melia azedarach* L. extracts. Homogenates were prepared and assayed as described in Material and Methods. A – aqueous extract; B – potassium phosphate buffer (pH 7.2) extract; C – hydroethanolic 70:30 extract; D – hydroethanolic 50:50 extract. ⁎ Control; ♦ 0.5 mg.ml^{-1} extract; ▬ 1.0 mg.ml^{-1} extract; ▲ 2.0 mg.ml^{-1} extract.

hard to quantify each component separately.

A second important observation can be drawn from extract preparation. An extraction produces non phenolic substances which may interfere during the flavonoids determination, contributed for high levels of quercetin equivalents. This could be linked to the absorption spectra revealed by hidroethanolic extracts in comparison to the spectra by aqueous and potassium phosphate buffer. Also, interaction between extracts components could be influencing these results. More detailed investigations could add to these interactions, to include also the characterization of the extracts constituents.

These results suggest that *M. azedarach* compounds contribute significantly to the butyrylcholinesterase activity. However, these results showed the direct action of extracts on this enzyme. The butyrylcholinesterase activity measurements were immediately after addition of extracts into the reaction mixture revealed direct interaction. In order to confirm the inhibitory effect of this plant, there is need for these extracts to be available on butyrylcholinesterase under cellular conditions.

Conclusion

The present work demonstrates that very low concentrations of *M. azedarach* extracts caused inhibitory effect on butyrylcholinesterase activity. On the other hand, this interaction between *M. azedarach* and active site butyrylcholinesterase is unclear. The effect on activity enzymatic could be the consequence of the diversity of compounds, in special flavonoids. Therefore, these results shown potential effect of *M. azedarach* extracts on the basis of the *in vitro* studies.

In vivo studies are needed to further confirm the effects extracts on the butyrylcholinesterase activity. Moreover, studies of the identified compounds from *M. azedarach* on the mechanism of inhibition properties are required.

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REFERENCES

Alche LE, Ferek GA, Meo M, Coto CE, Maier MS (2002). An antiviral meliacarpin from leaves of *Melia azedarach* L. Z. Naturforsch, 58: 215-219.

Barbosa-Filho JM, Vasconcelos THC, Alencar AA, Batista LM, Oliveira RAG, Guedes DN, Falcão HS, Moura MD, Diniz MFFM, Modesto-Filho J (2005). Plants and their active constituents from South, Central and North America with hypoglycemic activity. Rev. Bras. Farmacogn., 15: 392-413.

Carmona GN, Jufer RA, Goldberg SR, Gorelick DA, Greig NH, Yu QS, Cone EJ, Schindler CW (2000). Butyrylcholinesterase accelerates cocaine metabolism: *in vitro* and *in vivo* effects in nonhuman primates and humans. Drug Metab. Dispos., 28(3): 367-371.

Chaturvedi P, Segale M (2007). Effects of different types of water decoctions of fruit of *Melia azedarach* on glucose induced hyperglycemia, liver transaminases, lipid peroxidation and reduced glutathione in normal albino rats. Sci. Res. Essays, 2(9): 384-389.

Chiffelle I, Huerta AF, Lizana DR (2009). Physical and chemicals characterization of *Melia azedarach* L. fruit and leaf for use as botanical insecticide. Chilean J. Agric. Res., 69(1): 38-45.

Cimasoni G (1966). Inhibition of cholinesterases by fluoride *in vitro.* Biochem., J. 99: 133-137.

Çokugras AN (2003). Butyrylcholinesterase: Structure and physiological importance. Turk J. Biochem., 28(2): 54-61.

Darvesh S, Hopkins DA, Geula C (2003). Neurobiology of butyrylcholinesterase. Nat. Rev. Neurosci., 4:131-138.

Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem., 97: 654-660.

Ellman GL, Courtney KD, Andres V, Featherstone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-95.

Gao X, Ohlander M, Jeppsson N, Björk L, Trajkovski V (2000). Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea Buckthorn (*Hippophae rhamnoides* L.) during maturation. J. Agric. Food Chem., 48: 1485-1490.

Gutzeit HO, Tokalov SV, Ludwig-Muller J, Rusak G (2005). Monitoring flavonoid metabolism in human cells by exploiting fluorescence elicited upon quercetin/protein interactions. Croat. Chem. Acta., 78: 337-342.

Hanifa MSA, Al-Khatib MH (1984). Effect of *Melia azedarach* fruits on gipsing-restraint stress-induced ulcers in rats. Jpn. J. Pharmacol., 36: 527-533.

Jorge TCM, Lenartovicz V, Andrade MW, Bonafin T, Giordani MA, Bueno NBC, Schneider SLG (2009). Pediculicidal activity of hydroethanolic extracts of *Ruta graveolens, Melia azedarach* and *Sambucus australis*. Lat. Am. J. Pharm., 28(3): 457-459.

Katalinić M, Rusak G, Barović JD, Sinko G, Jelić D, Antolović R, Kovarik Z (2010). Structural aspects of flavonoids as inhibitors of human butyrylcholinesterase. Eur. J. Med. Chem., 45(1): 186-192.

Kim JY, Lee WS, Kim WS, Curtis-Long MJ, Lee BW, Ryu YB, Park KH (2011). Isolation of cholinesterase-inhibiting flavonoids from *Morus ihou*. J. Agric. Food Chem. 59(9): 4589-4596.

Lopes TJ, Xavier MF, Quadri MGN, Quadri MB (2007). Anthocyanins: A brief review of structural characteristics and stability. R. Bras. Agrociência, 13(3): 291-297.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with Folin phenol reagent. J. Biol. Chem., 193: 265-272.

Lucić A, Bradamante V, Radic B, Peraica M, Domijan AM, Fuchs R, Stavljenić-Rukavina A (2002). The effect of dichlorvos treatment on butyrylcholinesterase activity and lipid metabolism in rats. Arh. Hig. Rada Toksikol., 53: 275-282.

Menichini F, Tundis R, Loizzo MR, Bonesi M, Marrelli M, Statti GA, Menichini F, Conforti F (2009). Acetylcholinesterase and butyrylcholinesterase inhibition of ethanolic extract and monoterpenes from *Pimpinella anisoides* V Brig. (Apiaceae). Fitoterapia. 80: 297-300.

Michelin DC, Iha SM, Rinaldo D, Sannomiya M, Santos LC, Villegas W, Salgado HRN (2005). Antimicrobial activity of *Davilla elliptica* St. Hill (Dilleniaceae). Rev. Bras. Farmacog., 15(3): 209-211.

Nath MC, Chakravorty MK, Chowdhury SR (1946). Liebermann-Buchard reaction for steroids. Nature. 157: 103-104.

Nyunja ARO, Onyango JC, Erwin B (2009). The Kakamega forest medicinal plants resources and their utilization by the adjacent Luhya community. Int. J. Trop. Med., 4(3): 82-90.

Phua DH, Tsai WJ, Ger J, Deng JF, Yang CC (2008). Human *Melia azedarach* poisoning. Clin. Toxicol., 46: 1067-1070.

Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx M, Cazin M, Cazin JC, Bailleul F, Trotin F (2000). Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. J. Ethnopharmacol., 72(1-2): 35-42.

Sahu-Vinod K, Raghuveer I, Alok Shashi, Himanshu G (2010). Phytochemical investigation and chromatographic evaluation of the ethanolic extract of whole plant extract of *Dendrophthoe falcata* (L.F.) Ettingsh. I.J.P.S.R., 1(1): 39-45.

Samudram P, Rajeshwari H, Vakusi R, Geetha A, Sathiya Moorthi P (2008). Hepatoprotective activity of bi-herbal ethanolic extract on CCl_4 induced hepatic damage in rats. Asian J. Biochem., 3(5): 308-314.

Senol FS, Orhan I, Yilmz G, Ciçek M, Sener B (2010). Acetylcholinesterase, butyrylcholinesterase and tyrosinase inhibition studies and antioxidant activities of 33 *Scutellaria* L. taxa from Turkey. Food Chem. Toxicol., 43(3): 781-788.

Senthil-Nathan S, Chung PG, Murugan K (2005). Effect of biopesticides applied separately or together on nutritional indices of the rice leaffolder *Cnaphalocrocis medicinalis* (Guenée) (Lepidoptera: Pyralidae). Phytoparasitica. 33(3): 187-195.

Simmonds MSJ, Simpson SJ, Blaney WM (1992). Dietary selection behavior in *Spodoptera littoralis:* the effects of conditioning diet and conditioning period on neural responsiveness and selection behavior. J. Exp. Biol., 162: 73-90.

Van den Berg R, Haenen GRMM, Van den Berg H, Van den Berg V, Blast A (2000). The predictive value of the antioxidant capacity of structurally related flavonoids using the Trolox equivalent antioxidant capacity (TEAC) assay. Food Chem., 70(3): 391-395.

Vieira OMC, Santos MH, Silva GA, Siqueira AM (2005). Antimicrobial activity of *Struthanthus vulgaris* (erva-de-passarinho). Braz. J. Pharmacgnon., 15(2): 149-154.

Yatsuda R, Rosalen PL, Cury JA, Murata RM, Rehder VLG, Melo LV, Kooc H (2005). Effects of Mikania genus plants on growth and cell adherence of mutans streptococci. J. Ethnopharmacol., 97: 183-189.

Wall ME, Krider MM, Krewson CF, Eddy CR, Willaman JJ, Corell DS, Gentry HS (1954). Steroidal sapogenins. VII. Survey of plants for steroidal sapogenins and other constituents. J. Am. Pharm. Assoc., 43(1): 1-7.

Widmark EMP (1964). Blood alcohol. In: E. Merck AG (ed) Chemical methods of medical investigation. Tenth Edition, Germany. Pp. 72-75.