

Supplementary file

Effects of *Artemisia macrocephala* Jacquem on Memory Deficits and Brain Oxidative Stress in Streptozotocin Induced Diabetic Mice

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Supplementary information

Table S1. Cholinesterase (AChE and BuChE) inhibitory activity of crude extract and fractions.

Sample Test	Conc. (µg/mL)	AChE		BChE	
		% Inhibition	IC ₅₀ (µg/mL)	% Inhibition	IC ₅₀ (µg/mL)
Crd-Am	6.25	25.09±0.91	116.36±1.48	20.60±0.93	240.52±1.35
	15.5	23.61±1.15		17.19±1.44	
	31.25	34.10±1.21		27.09±1.25	
	62.5	45.61±1.09		35.77±1.31	
	125	53.71±1.48		43.12±1.49	
	250	57.14±1.33		51.97±1.35	
	500	65.09±1.67		57.12±1.40	
	1000	69.24±1.41		61.43±1.33	
nhex-Am	6.25	09.96±0.76	934.75±1.21	13.77±0.83	509.26±1.14
	15.5	13.07±0.92		17.96±1.09	
	31.25	17.11±1.51		21.89±1.40	
	62.5	21.29±1.38		34.12±1.37	
	125	25.56±1.27		39.09±1.24	
	250	39.20±1.51		43.72±1.38	
	500	46.51±1.44		49.09±1.14	
	1000	53.49±1.21		54.12±1.61	
Chl-Am	6.25	29.88±1.03	52.68±1.09	26.12±0.99	57.45±1.39
	15.5	35.21±1.13		31.90±1.11	
	31.25	38.52±1.21		35.61±1.28	
	62.5	59.31±1.09		54.39±1.39	
	125	63.44±1.12		60.91±1.30	
	250	73.79±1.29		70.62±1.44	
	500	77.22±1.44		73.19±1.51	
	1000	86.91±1.38		80.14±1.34	
Et-Am	6.25	25.87±1.11	75.19±1.02	23.66±1.03	116.58±1.09

	15.5	30.93±1.09		31.08±0.85	
	31.25	35.10±0.96		35.86±1.01	
	62.5	41.56±1.02		45.57±1.14	
	125	61.41±1.17		53.61±1.09	
	250	63.60±1.21		57.22±1.36	
	500	70.23±1.19		67.08±1.29	
	1000	78.12±1.33		74.21±1.48	
But-Am	6.25	19.11±1.19	254.66±1.34	13.45±0.91	265.33±1.41
	15.5	25.71±1.30		20.97±1.17	
	31.25	30.12±1.22		26.28±1.29	
	62.5	42.11±1.42		43.73±1.45	
	125	44.43±1.50		43.98±1.35	
	250	49.09±1.34		47.11±1.41	
	500	57.12±1.41		56.30±1.33	
	1000	67.61±1.39		62.19±1.49	
Aq-Am	6.25	15.60±0.93	502.71±1.28	14.72±0.98	513.66±1.33
	15.5	19.67±1.22		17.96±1.25	
	31.25	25.29±1.25		24.11±1.41	
	62.5	32.76±1.37		29.61±1.30	
	125	34.81±1.20		32.97±1.21	
	250	42.87±1.39		41.96±1.37	
	500	49.73±1.28		48.67±1.33	
	1000	56.12±1.33		54.83±1.29	
Donepezil	6.25	61.98±0.67	5.04±0.67	77.89±0.71	4.01±0.71
	15.5	73.67±1.01		86.41±1.13	
	31.25	81.28±1.19		90.12±1.29	
	62.5	85.12±1.31		95.80±1.47	
	125	93.88±1.49		98.16±1.40	
	250	97.12±1.41		99.08±1.37	
	500	97.27±1.39		99.10±1.35	
	1000	98.10±1.53		99.77±1.30	

All values are expressed as Mean ± SEM.

Table S2. Effect of crude extract and fractions of *A. macrocephala* on mice in novel object recognition test (NORT) in short term memory.

Treatment/Dose	Sample Phase		Test Phase		DI (%)
	Identical Object	Identical Object	Novel Object	Familiar Object	
	A1	A2	A1	A2	
Control	18.98±0.63	18.31±0.73	18.71±0.45	16.98±0.53	52.42±1.88
Diabetic	8.97±0.70	7.79±0.86	6.39±0.52 ^{###}	13.01±0.71	32.93±1.91
Crd-Am	100mg	14.71±0.78	14.97±0.80	14.31±0.68 ^{**}	55.81±1.76
	200mg	15.11±0.46	15.03±0.78	14.39±0.79 ^{**}	57.21±1.54
nhex-Am	75mg	14.89±0.72	15.31±0.56	10.76±0.68 [*]	44.74±1.56
	150mg	14.83±0.83	14.87±0.70	10.91±0.91 ^{**}	45.13±1.92
Chl-Am	75mg	14.91±0.84	15.01±0.77	14.54±0.81 ^{***}	60.86±1.81
	150mg	14.73±0.91	15.12±0.52	15.05±0.48 ^{***}	62.03±1.98
Et-Am	75mg	14.91±0.75	14.88±0.73	14.43±0.81 ^{**}	57.76±1.71
	150mg	14.86±0.66	14.93±0.79	14.22±0.90 ^{**}	58.16±1.96
But-Am	75mg	14.96±0.75	14.99±0.50	11.56±0.53 [*]	46.85±1.77
	150mg	14.83±0.77	15.08±0.84	11.39±0.67 [*]	46.18±1.69
Aq-Am	75mg	14.81±0.66	14.72±0.81	11.65±0.86 ^{**}	48.26±1.73
	150mg	15.09±0.70	15.04±0.56	11.48±0.74 [*]	47.07±1.78
Donepezil	2mg	14.98±0.90	14.64±0.61	14.92±0.84 ^{***}	64.09±2.01
Metformin	50mg	14.69±0.83	15.08±0.56	15.07±0.79 ^{***}	62.03±1.87

All values are expressed as Mean±SEM (n=6). Oneway ANOVA after which Dunnett's post hoc multiple comparison test. ^{###}P<0.001 comparison of STZ treated diabetic (amnesic) group vs. normal control, ^{*}P<0.05, ^{**}P<0.01 and ^{***}P<0.001 as comparison of STZ treated diabetic (amnesic) group vs. Donepezil, Metformin, crude extract and fractions-treated groups, using one way ANOVA followed by Dunnett comparison. Crude extract (Crd-Am), hexane (nhex-Am), chloroform (Chl-Am), ethyl acetate (Et-Am), butanol (But-Am) and aqueous (Aq-Am) fraction.

Table S3. Effect of crude extract and fractions of *A. macrocephala* on mice in novel object recognition test (NORT) in long term memory.

Treatment/Dose	Sample Phase		Test Phase		DI (%)
	Identical Object	Identical Object	Novel Object	Familiar Object	
	A1	A2	A1	A2	
Control	20.30±0.93	21.81±0.84	15.95±0.79	11.68±0.78	57.72±1.91
Diabetic	13.08±0.68	11.41±0.88	6.26±0.52 ^{###}	13.28±0.69	32.03±1.83
Crd-Am	100mg	18.43±0.87	16.73±0.79	14.85±0.87 ^{**}	56.72±1.89
	200mg	17.23±0.88	19.06±0.72	17.01±0.77 ^{***}	58.51±2.05
nhex-Am	75mg	18.31±0.97	16.64±0.89	10.74±0.68 ^{**}	46.63±1.88
	150mg	17.06±0.62	19.40±0.62	10.99±0.89 ^{***}	45.32±1.91
Chl-Am	75mg	18.50±0.70	16.65±0.74	17.42±0.77 ^{**}	63.39±2.08
	150mg	17.56±0.67	19.02±0.58	19.21±0.89 ^{***}	64.07±2.11
Et-Am	75mg	18.35±0.81	16.83±0.70	16.70±0.72 ^{**}	59.53±1.97
	150mg	17.30±0.96	19.16±0.78	17.71±0.69 ^{***}	60.98±1.89
But-Am	75mg	18.53±0.84	16.78±0.84	11.06±0.57 ^{**}	47.73±1.82
	150mg	17.40±0.79	19.22±0.81	11.39±0.67 ^{***}	48.06±1.88
Aq-Am	75mg	18.47±0.87	16.76±0.70	11.65±0.63 ^{**}	48.93±1.85
	150mg	17.36±0.76	19.13±0.60	12.05±0.67 ^{***}	49.87±1.79
Donepezil	2mg	16.45±0.83	17.95±0.63	23.94±0.84 ^{***}	66.11±1.97
Metformin	50mg	18.23±0.88	17.13±0.68	17.12±0.87 ^{**}	62.52±2.01

Mean ± SEM (n=6). Oneway ANOVA after which Dunnett's post hoc multiple comparison test. ^{###}P<0.001 comparison of STZ treated diabetic (amnesic) group vs. normal control, *P<0.05, **P<0.01 and ***P<0.001 as comparison of STZ treated diabetic (amnesic) group vs. Donepezil, Metformin, crude extract and fractions-treated groups, using one way ANOVA followed by Dunnet comparison. Crude extract (Crd-Am), hexane (nhex-Am), chloroform (Chl-Am), ethyl acetate (Et-Am), butanol (But-Am) and aqueous (Aq-Am) fraction.

Table S4. Effect of Crd-Am and fractions on AChE and ACh level in brain.

Sample Test		AChE	ACh
		(μmoles of substrate hydrolysed / min / g tissue)	(mmol/min/mg protein)
Control		12.01±1.13	16.21±1.33
Diabetic		28.92±1.23 ^{\$\$\$}	6.17±1.19 ^{##}
Crd-Am	100mg	16.11±1.08 [*]	12.87±1.21 [*]
	200mg	15.09±1.15 ^{**}	13.33±1.26 ^{**}
Chl-Am	75mg	14.01±1.01 ^{***}	13.48±1.22 ^{**}
	150mg	13.61±1.34 ^{***}	13.92±1.45 ^{***}
Et-Am	75mg	15.03±1.12 ^{**}	13.09±1.39 ^{**}
	150mg	14.98±1.27 ^{**}	13.37±1.25 ^{**}
Donepezil	2mg	12.38±1.36 ^{***}	15.08±1.44 ^{***}
Metformin	50mg	11.92±1.19 ^{***}	15.17±1.51 ^{***}

Mean±SEM (n = 6). Oneway ANOVA after which Dunnett's post hoc multiple comparison test. ^{\$\$\$} P<0 001, ^{##}P<0 001 comparison of STZ treated diabetic (amnesic) group vs. normal control, *P<0.05, **P<0.01 and ***P<0.001 as comparison of STZ treated diabetic (amnesic) group vs. Donepezil, Metformin, crude extract and fractions-treated groups, using one way ANOVA followed by Dunnet comparison. Crude extract (Crd-Am), chloroform (Chl-Am) and ethyl acetate (Et-Am) fraction.