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APPENDIXS

APPENDIX A PERCENTAGE OF YIELD

The percentage of yield of the *V. cinerea* Less. extract was calculated by using the following equation:

$$\boxed{\% \text{ yield} = [\text{dried extract weight (g)} / \text{plant material sample weight (g)}] \times 100}$$

% yield of *Vernonia cinerea* Less. Extracts

$$\% \text{ yield} = [16.269 \text{ (g)} / 100 \text{ (g)}] \times 100$$

$$= 16.269$$

APPENDIX B NICOTINE ABSTINENCE SIGNS SCORE

Table 4 Total abstinence sign score of mice at the first day after nicotine withdrawal

Treatments	N	Mean	SEM
Control	6	102.33	12.03
Nicotine withdrawal	6	134.17	6.86
Mecamylamine	6	128.83	12.86
VE125	6	77.33	15.46
VE250	6	51.33	13.46
VE500	6	17.00	4.10

Table 5 Total abstinence sign score of mice at the 7th day after nicotine withdrawal

Treatments	N	Mean	SEM
Control	6	67.17	9.29
Nicotine withdrawal	6	121.33	6.64
Mecamylamine	6	122.67	14.80
VE125	6	79.50	4.52
VE250	6	52.50	15.92
VE500	6	50.17	15.97

Table 6 Total abstinence sign score of mice at the first day after nicotine withdrawal observed every 5 minutes

Treatments	N	Abstinence signs (Mean±SEM)					
		5^{th} minute	10^{th} minute	15^{th} minute	20^{th} minute	25^{th} minute	30^{th} minute
Control	6	16.67 ± 7.58	21.50 ± 4.97	18.00 ± 2.44	18.83 ± 3.73	17.67 ± 2.06	10.17 ± 3.56
Nicotine withdrawal	6	11.50 ± 2.35	24.67 ± 5.33	25.33 ± 3.63	26.50 ± 2.14	24.00 ± 1.86	21.67 ± 1.52
Mecamylamine	6	10.00 ± 1.27	19.83 ± 2.97	24.17 ± 3.40	25.33 ± 2.92	27.83 ± 4.71	20.00 ± 3.13
VE125	6	8.83 ± 2.17	19.33 ± 3.03	15.17 ± 3.57	11.33 ± 3.17	13.00 ± 3.72	9.67 ± 3.69
VE250	6	6.00 ± 1.53	6.50 ± 1.59	9.83 ± 2.36	8.83 ± 4.76	9.33 ± 3.88	10.83 ± 5.08
VE500	6	6.00 ± 1.48	6.50 ± 3.21	3.33 ± 1.26	0.83 ± 0.54	0.33 ± 0.33	0.0 ± 0.0

Table 7 Total abstinence sign score of mice at the 7th day after nicotine withdrawal observed every 5 minutes

Treatments	N	Abstinence signs (Mean±SEM)					
		5th minute	10th minute	15th minute	20th minute	25th minute	30th minute
Control	6	16.67 ± 7.58	21.50 ± 4.97	18.00 ± 2.44	18.83 ± 3.73	17.67 ± 2.06	10.17 ± 4.23
Nicotine withdrawal	6	11.50 ± 2.35	24.67 ± 5.33	25.33 ± 3.63	26.50 ± 2.14	24.00 ± 1.86	21.67 ± 1.98
Mecamylamine	6	10.00 ± 1.27	19.83 ± 2.97	24.17 ± 3.40	25.33 ± 2.92	27.83 ± 4.71	20.00 ± 2.41
VE125	6	8.83 ± 2.17	19.33 ± 3.03	15.17 ± 3.57	11.33 ± 3.17	13.00 ± 3.72	9.67 ± 3.40
VE250	6	6.00 ± 1.53	6.50 ± 1.59	9.83 ± 2.36	8.83 ± 4.76	9.33 ± 3.88	10.83 ± 2.81
VE500	6	6.00 ± 1.48	6.50 ± 3.21	3.33 ± 1.26	0.83 ± 0.54	0.33 ± 2.29	0.0 ± 4.47

Table 8 Locomotor activity of mice at the first day after nicotine withdrawal observed every minute

Treatments	N	Locomotor activity (Mean±SEM)				
		1st minute	2nd minute	3rd minute	4th minute	5th minute
Control	6	20.67 ± 5.56	15.00 ± 3.31	20.00 ± 3.18	18.17 ± 2.99	17.67 ± 4.23
Nicotine withdrawal	6	8.83 ± 3.59	8.00 ± 3.34	6.67 ± 3.78	6.83 ± 4.17	14.50 ± 1.98
Mecamylamine	6	34.17 ± 8.25	22.17 ± 5.84	18.00 ± 4.12	13.50 ± 4.55	18.50 ± 2.41
VE125	6	11.67 ± 3.15	13.83 ± 3.74	14.00 ± 4.07	13.50 ± 3.43	20.17 ± 3.40
VE250	6	18.17 ± 5.82	16.17 ± 6.30	17.33 ± 3.84	10.67 ± 2.73	8.17 ± 2.81
VE500	6	15.17 ± 6.84	14.00 ± 5.47	4.50 ± 2.47	2.67 ± 2.29	6.83 ± 4.47

Table 9 Locomotor activity of mice at the 7th day after nicotine withdrawal observed every minute

Treatments	N	Locomotor activity (Mean±SEM)				
		1st minute	2nd minute	3rd minute	4th minute	5th minute
Control	6	36.00 ± 6.37	33.5 ± 5.90	23.00 ± 4.39	29.50 ± 3.46	23.00 ± 2.71
Nicotine withdrawal	6	35.83 ± 3.93	28.00 ± 3.56	25.83 ± 4.61	30.67 ± 3.58	23.83 ± 2.34
Mecamylamine	6	20.5 ± 3.14	22.00 ± 4.11	17.17 ± 4.74	14.50 ± 4.89	11.00 ± 2.42
VE125	6	15.5 ± 4.65	17.17 ± 1.99	15.67 ± 3.92	10.50 ± 2.41	10.67 ± 3.99
VE250	6	15.83 ± 2.96	17.67 ± 2.91	18.83 ± 2.27	17.33 ± 3.38	10.50 ± 4.38
VE500	6	22.00 ± 6.30	18.17 ± 4.45	15.17 ± 2.27	10.33 ± 3.51	14.17 ± 4.22

Table 10 Open-arm entry of mice at the first day after nicotine withdrawal observed every minute

Treatments	N	Anxiety-like behavior (Mean±SEM)				
		1st minute	2nd minute	3rd minute	4th minute	5th minute
Control	6	1.17 ± 0.31	0.50 ± 0.22	0.33 ± 0.21	0.33 ± 0.33	0.50 ± 0.22
Nicotine withdrawal	6	1.33 ± 0.33	1.17 ± 0.40	1.33 ± 0.49	0.50 ± 0.22	1.00 ± 0.37
Mecamylamine	6	1.67 ± 0.56	0.50 ± 0.22	0.33 ± 0.21	0.50 ± 0.34	0.67 ± 0.21
VE125	6	1.67 ± 0.42	0.83 ± 0.31	0.50 ± 0.22	1.00 ± 0.45	0.83 ± 0.31
VE250	6	1.50 ± 0.50	0.83 ± 0.40	0.33 ± 0.21	0.17 ± 0.17	0.0 ± 0.0
VE500	6	1.67 ± 0.21	1.00 ± 0.52	0.50 ± 0.34	0.50 ± 0.34	0.33 ± 0.21

Table 11 Open-arm entry of mice at the 7th day after nicotine withdrawal observed every minute

Treatments	N	Anxiety-like behavior (Mean±SEM)				
		1st minute	2nd minute	3rd minute	4th minute	5th minute
Control	6	0.67 ± 0.49	0.83 ± 0.48	0.67 ± 0.33	1.17 ± 0.31	0.33 ± 0.21
Nicotine withdrawal	6	1.83 ± 0.75	0.83 ± 0.17	1.33 ± 0.21	1.00 ± 0.26	1.50 ± 0.67
Mecamylamine	6	0.67 ± 0.42	1.50 ± 0.43	0.83 ± 0.31	0.67 ± 0.33	0.83 ± 0.40
VE125	6	0.50 ± 0.22	1.50 ± 0.34	0.67 ± 0.33	1.50 ± 0.62	0.67 ± 0.21
VE250	6	1.83 ± 0.60	1.50 ± 0.50	1.50 ± 0.22	2.33 ± 0.76	1.50 ± 0.43
VE500	6	1.83 ± 0.31	1.17 ± 0.31	1.50 ± 0.43	0.83 ± 0.40	1.00 ± 0.37

Table 12 Percent time spent in open-arm entry of mice at the first day after nicotine withdrawal

Treatments	N	Mean	SEM
Control	6	4.89	1.59
Nicotine withdrawal	6	9.59	1.40
Mecamylamine	6	6.22	1.51
VE125	6	7.00	1.37
VE250	6	6.50	1.67
VE500	6	7.39	1.87

Table 13 Percent time spent in open-arm entry of mice at the 7th day after nicotine withdrawal

Treatments	N	Mean	SEM
Control	6	6.22	1.86
Nicotine withdrawal	6	9.39	2.54
Mecamylamine	6	9.28	2.15
VE125	6	8.61	1.36
VE250	6	15.72	3.68
VE500	6	11.00	1.59

Table 14 Close-arm entry of mice at the first day after nicotine withdrawal observed every minute

Treatments	N	Anxiety-like behavior (Mean±SEM)				
		1st minute	2nd minute	3rd minute	4th minute	5th minute
Control	6	4.83 ± 0.91	3.83 ± 0.60	2.50 ± 0.50	2.50 ± 0.56	2.17 ± 0.54
Nicotine withdrawal	6	4.50 ± 0.62	3.33 ± 0.56	2.50 ± 0.62	2.17 ± 0.70	2.33 ± 0.42
Mecamylamine	6	5.67 ± 0.21	4.50 ± 0.43	3.50 ± 0.43	3.00 ± 0.82	2.50 ± 0.34
VE125	6	4.33 ± 1.28	4.83 ± 0.48	3.00 ± 0.45	3.33 ± 0.72	2.00 ± 0.45
VE250	6	3.00 ± 0.45	3.00 ± 0.68	2.83 ± 0.75	2.67 ± 0.62	1.17 ± 0.31
VE500	6	3.17 ± 0.40	2.17 ± 0.65	2.00 ± 0.58	1.50 ± 0.22	1.83 ± 0.83

Table 15 Close-arm entry of mice at the 7th day after nicotine withdrawal observed every minute

Treatments	N	Anxiety-like behavior (Mean±SEM)				
		1st minute	2nd minute	3rd minute	4th minute	5th minute
Control	6	4.50 ± 0.34	2.83 ± 0.40	3.00 ± 0.45	2.50 ± 0.22	1.67 ± 0.33
Nicotine withdrawal	6	4.50 ± 0.56	3.33 ± 0.72	3.50 ± 0.85	2.67 ± 0.42	2.83 ± 0.65
Mecamylamine	6	4.50 ± 0.43	3.17 ± 0.31	3.00 ± 0.37	1.83 ± 0.65	1.83 ± 0.65
VE125	6	4.33 ± 0.56	4.50 ± 0.56	3.33 ± 0.42	3.67 ± 0.56	2.83 ± 0.31
VE250	6	6.00 ± 0.68	5.00 ± 0.0	4.33 ± 0.49	3.33 ± 0.42	2.67 ± 0.62
VE500	6	6.00 ± 0.68	3.33 ± 0.42	3.50 ± 0.22	3.67 ± 0.76	2.83 ± 0.17

APPENDIX C RECEPTOR DISPLACEMENT ASSAYS

Receptor displacement activity was calculated by using the following equations

For nicotinic receptor displacement activity

fMol bound/mg protein = [(Total binding (CPM) – nonspecific binding (CPM)) /155.265] / mg protein

For nicotinic receptor displacement activity

fMol bound/mg protein = [(Total binding (CPM) – nonspecific binding (CPM)) / 164.859] / mg protein

Table 16 Data of nicotinic receptor displacement assay

Treatments (mg/ml)	N	CPM			fMol bound/mg protein			Mean	SEM
		1	2	3	1	2	3		
Control	3	226.00	365.00	235.67	1.17	3.75	6.30	3.74	1.48
0.05	3	259.00	311.33	251.33	2.59	1.45	6.97	3.67	1.68
0.1	3	207.67	347.00	254.67	0.39	2.98	7.11	3.49	1.96
0.25	3	206.33	315.33	223.00	0.33	1.62	5.75	2.57	1.64
0.5	3	207.33	258.33	205.67	0.37	-0.83	5.01	1.52	1.78
1.0	3	163.00	228.33	226.00	-1.53	-2.12	5.88	0.74	2.57
2.5	3	155.67	215.00	161.67	-1.85	-2.69	3.12	-0.47	1.81
5.0	3	108.00	144.33	168.67	-3.89	-5.73	3.42	-2.07	2.79
10.0	3	126.33	208.67	82.67	-3.11	-2.96	-0.27	-2.11	0.92
Non-specific binding	3	198.67	277.67	89.00	0.0	0.0	0.0	0.0	0.0

Table 17 Data of muscarinic receptor displacement assay

Treatments (mg/ml)	N	CPM			fMol bound/mg protein			Mean	SEM
		1	2	3	1	2	3		
Control	3	2280.67	2721.67	2594.67	84.97	102.66	100.10	95.91	5.52
0.05	3	2196.67	2305.33	1881.00	81.58	85.82	71.24	79.55	4.33
0.1	3	2036.33	2066.00	1891.67	75.09	76.15	71.67	74.30	1.35
0.25	3	2232.00	2332.33	2448.67	83.01	86.92	94.19	88.04	3.28
0.5	3	2119.33	2308.00	2407.33	78.45	85.93	92.52	85.64	4.06
1.0	3	1860.33	1756.33	1700.33	67.98	63.62	63.93	65.18	1.40
2.5	3	1126.33	1392.33	1205.00	38.30	48.90	43.90	43.70	3.06
5.0	3	770.33	992.67	1074.33	23.90	32.74	38.62	31.75	4.28
10.0	3	428.00	687.67	370.00	10.06	20.41	10.14	13.53	3.44
Non-specific binding	3	179.33	183.00	119.33	0.0	0.0	0.0	0.0	0.0

APPENDIX D WESTERN BLOT ASSAYS

Table 18 Data of nicotinic receptor expression by western blot assay

Treatments	N	Band intensity				Mean	SEM
		1	2	3	4		
Control	4	190.56	183.64	203.17	202.02	194.85	4.70
Nicotine withdrawal	4	185.83	181.30	206.98	206.73	195.21	6.79
Mecamylamine	4	200.47	187.81	207.21	211.82	201.83	5.22
VE125	4	188.76	185.41	215.61	186.37	194.04	7.23
VE250	4	203.22	185.37	207.41	193.45	197.36	4.95
VE500	4	184.18	163.92	211.67	194.13	188.47	9.96

Table 19 Data of muscarinic receptor expression by western blot assay

Treatments	N	Band intensity				Mean	SEM
		1	2	3	4		
Control	4	190.56	183.64	203.17	202.02	164.04	14.64
Nicotine withdrawal	4	185.83	181.30	206.98	206.73	161.59	15.56
Mecamylamine	4	200.47	187.81	207.21	211.82	149.52	10.84
VE125	4	188.76	185.41	215.61	186.37	163.23	17.04
VE250	4	203.22	185.37	207.41	193.45	156.58	14.81
VE500	4	184.18	163.92	211.67	194.13	155.65	13.14

APPENDIX E AUTORADIOGRAPHIC ASSAYS

Table 20 Data of muscarinic receptor expression by autoradiographic study

Groups	fmol/mg		
	Nucleus accumbens	**Hippocampus**	**Ventral tegmental area**
Control-1	598.03	481.46	244.26
Control-2	561.39	481.53	235.68
Control-3	453.12	521.27	429.88
Nicotine withdrawal-1	603.68	413.50	327.35
Nicotine withdrawal-2	572.98	335.62	286.07
Nicotine withdrawal-3	575.63	468.18	294.16
Mecamylamine-1	350.54	369.06	365.58
Mecamylamine-2	580.04	439.69	231.04
Mecamylamine-3	604.95	481.01	278.71
VE125-1	602.31	479.14	194.47
VE125-2	547.86	400.08	202.59
VE125-3	516.08	361.06	151.74
VE250-1	612.82	420.11	199.72
VE250-2	664.24	450.09	222.94
VE250-3	498.75	462.32	197.40
VE500-1	582.42	451.37	158.11
VE500-2	518.58	401.01	150.01
VE500-3	615.80	386.83	211.37

BIOGRAPHY

BIOGRAPHY

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