

The Addictive and Neurotoxicological Evaluation of Cannabis sativa, male Carica papaya, Nicotiana tabacum and Datura stramonium Alkaloid Extracts via Neuroinflammatory, Neurotransmitter and Oxidative Systems

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Abstract

Drug addiction is a relapsing and chronic brain ailment that is accompanied by habitual, uncontrollable and pathological pattern of substance seeking and usage despite the associated severe negative social and health consequences. This study evaluated the addictive and neurotoxicological evaluation of *Cannabis sativa*, male *Carica papaya*, *Nicotiana tabacum* and *Datura stramonium* alkaloid extracts via neuroinflammatory, neurotransmitter and oxidative systems. For 90 days, the alkaloid extracts were orally administered to experimental rats and neurobehavioral paradigms were evaluated on day 91. Rats were sacrificed and striatum homogenate prepared. Expression of addiction and neurotoxicity-related genes, alongside biochemical neurotransmitter and cytokine metabolisms were evaluated. Result established the addictive and neurotoxicological potentials of the alkaloid extracts via behavioural paradigms, coupled with inflammatory, monoaminergic, apoptotic, cholinergic, oxidative and glutamatergic neurotransmission systems modulations. However, observed neurotoxicity of the psychoactive plants' alkaloids was not directly proportional to their addictiveness as the psychoactive plants ranked AECS > AENT > AEDS > AECM in addictiveness but ranked AEDS > AENT > AECS > AECM for toxicological potentials, as measured using related behavioural, neurotransmitter, apoptotic and inflammatory systems. Conclusively, the toxicological effects of the psychoactive plants' alkaloids are mostly expressed at high doses.

Introduction

In lieu of feeding from natural abundance, primitive human incidentally discovered plants with addictive properties, of which their abuse has resulted in a global burden that has persisted till today (Fasakin et al. 2021). Drug addiction is a relapsing and chronic brain ailment that is accompanied by habitual, uncontrollable and pathological pattern of substance seeking and usage despite the associated severe negative social and health consequences. An estimation of about 10% of the global population are at risk of drug addiction, which may be as high as 21.28% and 14.4% in the United States of America and Nigeria respectively (Lamontagne and Olmstead, 2018; UNODC 2018; 2019; Citaristi, 2022). Drug addiction leads to neurological impairments and prioritizing of recreational use of psychoactive substances over life-sustaining activities and goals. It results into long- and short-term social, neurological and health problems. The death of about 1.8 million individuals is also reported every year (Cunha-Oliveira et al., 2008; Roth et al. 2018; Fasakin et al. 2022a). Regrettably, the highest proportion of global population addicted to psychoactive substance usage are the youths, the future of every society (Nnam et al., 2021). To further exacerbate the problem, addiction victims rarely seek or continue with treatment (Lamontagne and Olmstead, 2018).

Plants with these addictive properties are known as psychoactive plants, with examples including *Cannabis sativa*, male *Carica papaya*, *Nicotiana tabacum* and *Datura stramonium*, which have been surveyed to elicit 13.8–22.4% and 10.0–14.9% prevalence of recreational use in the southern and northern geopolitical zones of Nigeria respectively (Fasakin et al. 2022b). Typically, the alkaloids of the psychoactive plants have been implicated in their psychoactive, reinforcing and rewarding effects (Jean-Francois, 2014; Fasakin et al., 2021). In addition to pleasure incentives, the perception that the plants' constituents can enhance sexual performance, alleviate anxiety, depressive moods, and hypertensive conditions (Fasakin et al. 2022a,b,c), have further escalated the plants' prevalent use, as the treated-individuals still continue the use of the plants after recovery from the treated-ailment (Müller et al. 2020). Addiction to these psychoactive plants have been observe to come in diverse biological phases (intoxication, withdrawal, bingeing, craving) and each of the phases involves the

interaction of the plants' phytoconstituents with several brain structures and circuits thereby altering the response of brain systems to psychological stimuli (food, social interaction, water, etc.) key to existence (Cunha-Oliveira et al. 2013; Fasakin et al. 2022b).

The chronic usage of these psychoactive plants has been suggested to evoke neurotoxicity via aggravation of synaptic dopaminergic (reward and nigrostriatal) and extra-synaptic glutamate concentrations, characterized by other neurotransmitter systems' alterations, apoptosis, reactive species generation, neurocognitive impairments and energy metabolism dysregulation (Fasakin et al. 2022b; Niedzielska-Andres et al. 2022). Also, the dopaminergic and glutamatergic neuronal pathways that mediate the addictive and neurotoxicological properties of psychoactive substances are domicile in the striatum, which has been established to be more sensitive to induced-neurotoxicity than the nucleus accumbens (Itzhak and Ali, 2002; Yager et al., 2015), therefore, the present study will be evaluating the striatum for the addictive and neurotoxicological potentials of the psychoactive plants. Also, the higher prevalence in the recreational use of psychoactive substances observed in men (21.8%) compared to women (7.0%) (UNODC, 2018), underlies the use of male rats.

Materials and methods

Chemical and reagents

Acetylthiocholine iodide, adenosine, adenosine monophosphate, Ellmans' reagent, were procured from Sigma-Aldrich. VWR life science (Solon, United States of America) and Zymo Research (United States of America) supplied TRI Reagent® and Nuclease-free water, respectively. Inqaba Biotechnology (Hatfield, South Africa) supplied the primers used in the present study.

Sample collection and extracts preparation

Leaves of *Cannabis sativa*, male *Carica papaya* and *Nicotiana tabacum*, and *Datura stramonium* seeds were procured. They were then authenticated by the Centre for Research and Development (CERAD) unit of the Federal University of Technology, Akure (FUTA), Nigeria. Fasakin et al. (2021) procedure was employed in alkaloid extracts preparation. Resultant alkaloid extracts were then termed AECS (alkaloid extract of *Cannabis sativa*), AENT (alkaloid extract of *Nicotiana tabacum*), AEDS (alkaloid extract of *Datura stramonium*), and AECM (alkaloid extract of *Carica papaya* male).

Experimental protocol

Adult male Wistar albino rats (weighting 167 to 185 g) were procured for the present study. The male Wistar rats were contained in plastic cages with relative humidity (60 to 70%), controlled light of 12 h light and dark daily, and room temperatures (25 to 27 °C) (Public Health Service 1996; Oyeleye et al. 2022). The rats were handled and used as permitted by the Centre for Research and Development (CERAD) Animal Ethical Committee of FUTA (FUTA/ETH/2020/016). Six experimental animals per group were then randomly selected, sectioned and once daily administered alkaloid extracts as previously described by Fasakin et al. (2022a):

Group 1 comprised of normal experimental rats.

Group 2, 6, 10 and 14 received 5 mg/kg of AECS, AEDS, AENT, AECM, respectively.

Group 3, 7, 11 and 15 received 50 mg/kg of AECS, AEDS, AENT, AECM, respectively.

Group 4, 8, 12 and 16 received 500 mg/kg of AECS, AEDS, AENT, AECM, respectively.

Group 5, 9, 13 and 17 received 2000 mg/kg of AECS, AEDS, AENT, AECM, respectively.

Behavioural tests

Alkaloid extracts administered rats were then exposed to behavioural tests (conditioned place preference, Y-Maze and Morris Water Maze) to establish the addictive and neurotoxicological potentials of the alkaloid extracts during oral administration. Experimental apparatuses were thoroughly cleaned with isopropyl alcohol after each animal session to remove olfactory cues.

Conditioned Place Preference (CPP) Test

CPP was conducted to assess the addictive potentials of the extracts (Itzhak and Ali 2002; Möller et al. 2018). The CPP apparatus comprised of two compartments of 30 (height) x 20 (width) x 20 (length) with different visually (stripes/plain) coloured walls and textured (rough/smooth) floor designs separated by a removable guillotine door. The CPP paradigm comprised of three different stages of habituation (subjection of experimental animals to CPP apparatus prior to commencing experiment to determine drug-paired [least time spent] and vehicle-paired [highest time spent] compartments); conditioning (daily subjection of normal control and alkaloid extract exposed-animals to vehicle-paired and drug-paired compartments after administration); and post-conditioning (evaluation of addictiveness by placing experimental rat in vehicle-paired section and allowed free entrance to both section, a day after the last administration) stages for 20 minutes each per session, which was video-recorded under a dim white dispersed light and analysed.

Y-Maze Test

Y-maze test was conducted to measure the memory index (percentage alteration) of the alkaloid extracts exposed rats using the method of Ademosun et al. (2022).

Morris Water Maze (MWM) Test

MWM test was carried out to assess the cognitive function of the alkaloid extracts exposed rats (Oyeleye et al. 2022).

Tissue homogenate preparation

On day 91 of the experiment, alkaloid extract administered-rats were anesthetized using mild diethyl ether, sacrificed and brain removed. Afterwards, the striatum was carefully removed for subsequent analysis (Fasakin et al. 2022a).

Gene expression analysis

Total ribonucleic acid was extracted from the striatal tissues using TRI Reagent® while relative cDNA amount was quantified as previously reported by Fasakin et al. (2022a) with β -actin gene used as standard (Table 1).

Table 1
Real-time quantitative PCR primer sequence

Gene	Sequence
β-actin	CTCCCTGGAGAAGAGCTATGA-Forward
	AGGAAGGAAGGCTGGAAGA-Reverse
N-methyl-D-aspartate receptor subunit 2A (NR2A)	AGCCCCCTTCGTCATCGTAGA-Forward
	ACCCCTTGCAGCACTTCTTCAC-Reverse
Nuclear Factor-kappa B	AGACATCCTTCCGCAAAC-Forward
	TAGGTCCATCCTGCCCATAA-Reverse
p53	ACGTGATGAGGATGGGAAAC-Forward
	TATCTGGCTTCTTGCTCTTGG-Reverse

Table 2
GC-MS characterization of AECS

S/N	RT(min)	Compound Name	Mol. Formula	MW	Peak Area %	Comp (µg/g)
1	10.82	Choline	C ₅ H ₁₄ NO ⁺	104	2.23	23.03
2	13.41	Trigonelline	C ₇ H ₇ NO ₂	137	3.59	20.41
3	14.00	Dopamine	C ₈ H ₁₁ NO ₂	153	7.18	86.09
4	16.28	Hordenine	C ₁₀ H ₁₅ NO	165	2.93	27.02
5	19.18	Cannabidiol	C ₂₁ H ₃₀ O ₂	448.2	50.08	193.12
6	20.01	Delta-9-tetrahydrocannabinol	C ₂₁ H ₃₀ O ₂	463.2	25.04	193.12
7	21.05	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	C ₁₀ H ₁₄ N ₂	162	4.31	331.77
8	29.00	Morphine	C ₁₇ H ₁₉ NO ₃	285	2.19	20.02
9	32.00	D-Lysergic acid N,N-diethylamide	C ₂₀ H ₂₅ N ₃ O	323	3.03	50.04
10	35.60	Boldine	C ₁₉ H ₂₁ NO ₄	327	5.75	103.41
11	41.50	Strychnine	C ₂₁ H ₂₂ N ₂ O ₂	334	8.62	72.63
12	43.00	Anhydrocannabisativine	C ₂₁ H ₃₇ N ₃ O ₂	363	12.25	218.11
13	44.31	Cannabisativine	C ₂₁ H ₃₉ N ₃ O ₃	381	17.24	410.30
14	46.78	Cannabimine C	C ₂₁ H ₃₇ N ₃ O ₂	363	5.78	376.12
15	48.80	Aconitine	C ₃₄ H ₄₇ NO ₁₁	645	7.54	160.43

Table 3
GC-MS characterization of AEDS

S/N	RT(min)	Compound Name	Mol. Formula	MW	Peak Area %	Comp (µg/g)
1	7.50	2-Pyrrolidinone	C ₄ H ₇ NO	85	6.15	110.24
2	7.81	8-Methyl-8-azabicyclo[3.2.1]octane-2,6-diol	C ₈ H ₁₅ NO ₂	157	1.02	10.06
3	12.00	Ecgonine ethyl ester	C ₁₁ H ₁₉ NO ₃	213	13.68	37.84
4	13.00	Benzeneacetic acid, α-methylene-, 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester, endo-	C ₁₇ H ₂₁ NO ₂	271	1.05	10.72
5	17.00	Dasycarpidan-1-methanol, acetate (ester)	C ₂₀ H ₂₆ N ₂ O ₂	326	16.39	314.60
6	18.11	Anisodine	C ₁₇ H ₂₁ NO ₅	319	4.10	208.14
7	21.50	Littorine	C ₁₇ H ₂₃ NO ₃	289	4.61	157.03
8	22.00	Hyoscyamine	C ₁₇ H ₂₃ NO ₃	289	6.14	262.21
9	23.98	Atropine	C ₁₇ H ₂₃ NO ₃	289	2.95	21.28
10	25.00	2,7-Diphenyl-1,6-dioxypyridazino[4,5:2,3]pyrrolo[4,5-d]pyridazine	C ₂₀ H ₄₀ O	355	9.32	341.41
11	27.50	17-(1,5-Dimethylhexyl)-10,13-dimethyl-4-vinylhexadecahydrocyclopenta[a]phenanthren-3-ol	C ₂₉ H ₅₀ O	414	7.17	110.43
12	28.50	3-(1,5-Dimethyl-hexyl)3a,10,10,12b-tetramethyl1,2,3,3a4,6,8,9,10,10a,11,12,12a,12b-tetradecahydro-benzo[4,5] Cyclohept	C ₁₀ H ₅₀	410	4.23	32.08
13	30.50	3,8,8-Trimethoxy-3-piperidyl-2,2-benaphthalene-1,1,4,4-tetrone	C ₂₈ H ₂₅ NO ₇	487	6.15	11.23
14	31.75	Ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	436	2.56	20.05
15	32.36	Scopolamine	C ₁₇ H ₂₁ NO ₄	303	3.65	60.40
16	40.48	[5β]Pregnane-3,20beta-diol, 14alpha,18alpha-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate	C ₂₉ H ₄₈ O	489	3.07	26.17
17	44.56	1-Monolinolein, 2TMS derivative	C ₃₆ H ₇₄	498	14.34	110.07

Biochemical analysis

Bradford (1976) method was employed to evaluate striatal protein. Green and Haughton (1961) method was employed to evaluate monoamine oxidase activity at 450 nm using 2, 4-Dinitrophenylhydrazine, 0.0125 mol/L semicarbazides, 0.1 N NaOH, 10 mmol/L benzylamines, acetic acid and benzene. Guo et al. (2009) method was

employed to evaluate striatal dopamine concentrations at 735 nm while acetylcholinesterase (AChE) activity was evaluated using AChE as substrate as reported by Perry et al. 2000. Abdel-Zaher et al. (2011) method was employed to evaluate glutamate dehydrogenase activity, while Hayashi et al. (2007) method was employed to quantify reactive oxygen species (ROS) production. Nitric oxide concentration was evaluated using Green et al. (1982) method. The activity of lactate dehydrogenase and concentrations of interleukin-1 β and 10, tumour necrosis factor- α were evaluated using commercially available Enzyme-Linked Immunosorbent Assay kits.

GC-MS analysis of AECS, AEDS, AENT and AECM

Qualitative classification analyses of possible compounds in the AECS, AEDS, AENT and AECM were carried out as previously reported by Fasakin et al. (2022a).

Statistical analysis

Kolmogorov–Smirnov test was employed to check obtained data for normality patterns while one-way analysis of variance was employed to analyse the data using GraphPad version 8.0.2. followed by Tukey's test. Results are expressed mean $\pm$ SEM ($n = 6$) and significantly different at $p < 0.05$

Results

GCMS Analysis of AECS, AEDS, AENT, and AECM

Result showed the GC–MS characterization (Fig. 1–4) and the alkaloid constituents (Tables 1–4) of AECS, AEDS, AENT, and AECM.

Table 4
GC-MS characterization of AENT

S/N	RT(min)	Compound Name	Mol. Formula	MW	Peak Area %	Comp (µg/g)
1	7.60	Pyridine, 3-(3,4-dihydro-2H-pyrrol-5-yl)-	C ₉ H ₁₀ N ₂	146	2.44	21.03
2	10.25	Anatabine	C ₁₀ H ₁₂ N ₂	160	13.43	746.50
3	11.00	Quinoline, 2-methyl-	C ₁₀ H ₉ N	143	8.67	IS
4	12.67	Nicotine	C ₁₀ H ₁₄ N ₂	162.23	7.94	570.36
5	16.00	Nicotyrine	C ₁₀ H ₁₀ N ₂	158	1.83	10.96
6	18.00	Nornicotyrine	C ₉ H ₈ N ₂	144	3.66	11.36
7	18.57	2,2'-Bipyridine	C ₁₀ H ₈ N ₂	156	6.10	13.59
8	20.98	Anabasine	C ₁₀ H ₁₄ N ₂	162	7.33	214.93
9	26.50	2,3'-Dipyridyl	C ₁₀ H ₈ N ₂	156	6.31	40.80
10	27.00	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	C ₁₀ H ₁₄ N ₂	162	15.87	1844.00
11	33.76	Cotinine	C ₁₀ H ₁₂ N ₂ O	176	3.05	14.90
12	34.21	N-Nitrososornicotine	C ₉ H ₁₁ N ₃ O	177	5.00	12.00
13	38.76	Nornicotine, N-formyl	C ₁₀ H ₁₂ N ₂ O	176	6.08	18.04
14	42.50	2-Pyrrolidinone, 3-hydroxy-1-methyl-5-(3-pyridinyl)-, (3R-trans)	C ₁₀ H ₁₂ N ₂ O ₂	192	7.39	10.00
15	46.50	Nornicotine, N-acetyl	C ₁₁ H ₁₄ N ₂ O	190	10.99	11.15

Table 5
GC–MS characterization of AECM

S/N	RT(min)	Compound Name	Mol. Formula	MW	Peak Area %	Comp (µg/g)
1	3.81	Choline	C ₅ H ₁₄ NO ⁺	104	0.88	7.04
2	5.25	8-Methyl-8-azabicyclo[3.2.1]octane-2,6-diol	C ₈ H ₁₅ NO ₂	157	0.97	8.05
3	11.18	Benzylthiourea	C ₈ H ₁₀ N ₂ S	166	0.99	8.53
4	12.25	3,3-Diethyl-5-methyl-piperidine-2,4,6-trione	C ₁₀ H ₁₅ NO ₃	197	1.16	10.29
5	13.27	Anonaine	C ₁₇ H ₁₅ NO ₂	265	1.18	10.02
6	13.50	Trigonelline	C ₇ H ₇ NO ₂	137	14.07	57.06
7	14.20	+)–Laurotetanine	C ₁₉ H ₂₁ NO ₄	327	12.40	120.54
8	15.76	Pseudocarpaine	C ₂₈ H ₅₀ N ₂ O ₄	478	10.55	412.92
9	16.25	Carpaine	C ₂₈ H ₅₀ N ₂ O ₄	478	19.27	514.36
10	16.50	Alstoniaphylline C	C ₂₀ H ₂₄ N ₂ O ₃	350	10.34	40.38
11	17.32	(+)–alstonisine	C ₂₀ H ₂₂ N ₂ O ₃	338	6.16	152.90
12	18.00	Dehydrocarpaine I	C ₂₈ H ₄₈ N ₂ O ₄	476	4.40	445.18
13	20.42	Dehydrocarpaine II	C ₂₈ H ₄₆ N ₂ O ₄	434	5.32	274.71

Neuromodulatory Effects of AECS, AEDS, AENT, and AECM using Rat Behavioural Models of Addiction and Neurotoxicity

The effects of the alkaloid extracts were evaluated for addiction and neurotoxicity using conditioned place preference (CPP), Y-maze and Morris water maze tests. Result showed that animals administered 5, 50 and 500 mg/kg bwt of AECS, AENT, and AECM expressed significant ($P < 0.0001$; $F_{(16,85)} = 631.3$) alternation in preference index during CPP tests (Fig. 5). The administration of AECS, AEDS, AENT and AECM elicited a biphasic response as shown in Fig. 5 after the administration of the extracts for 90 days. Obtained result showed significantly ($P < 0.0001$; $F_{(16,85)} = 305.9$) improved preference index for AECS, AENT and AECM, whereas negative result was obtained for AEDS at 500 and 2000 mg/kg bwt, which may be suggestive of aversiveness at the dosages. Also, the rats elicited biphasic response ($P < 0.0001$; $F_{(16,85)} = 128.7$) spatial working memory (Y-maze test) after the sub-chronic administration of AECS, AENT and AECM in comparison to control group (Fig. 6). The administration of AEDS depleted spontaneous alternations in comparison to control group, an indication of reduced spatial memory function. The same pattern was obtained for spatial learning and memory indexes during MWM test ($P < 0.0001$; $F_{(16,85)} = 248.5$) (Fig. 7). Typically, administration of AECS, AENT and AECM significantly ($P < 0.05$) reduced escape latency at lower doses, while AEDS administration significantly ($P < 0.05$) elevated escape

latency at all doses. Furthermore, all the alkaloid extracts administration resulted in significantly ($P < 0.0001$; $F_{(16,85)} = 248.5$) elevated escape latency at 2000 mg/kg bwt in comparison to control group.

Neuromodulatory Effects of AECS, AEDS, AENT, and AECM on Neurotransmitter Systems linked to Addiction and Neurotoxicity

The effect of sub-chronic exposure of the alkaloid extracts on neurotransmitter metabolism are showed in Fig. 8–13. Results showed that the activities of Glutamate dehydrogenase ($P < 0.0001$; $F_{(16,85)} = 94.41$), monoamine oxidase ($P < 0.0001$; $F_{(16,85)} = 55.88$), acetylcholinesterase ($P < 0.0001$; $F_{(16,85)} = 207.0$), E-NTPDase [ATP ($P < 0.0001$; $F_{(16,85)} = 197.1$) and ADP ($P < 0.0001$; $F_{(16,85)} = 152.6$)] and ecto-5'-nucleotidase ($P < 0.0001$; $F_{(16,85)} = 181.4$) were altered in the striatum of rats exposed to AECS, AEDS, AENT, and AECM (Fig. 8–13). Striatal dopaminergic concentrations showed a biphasic dose-response curve ($P < 0.0001$; $F_{(16,85)} = 81.63$), while there was a significant ($P < 0.0001$; $F_{(16,85)} = 98.86$) increase in striatal nitric oxide concentrations (Figs. 14 and 15). Interestingly, the concentration of NO was consistent with that observed for dopamine concentration, which is suggestive of the link between the two neurotransmission systems.

Neuromodulatory Effects of AECS, AEDS, AENT, and AECM on Inflammatory Markers linked to Addiction and Neurotoxicity

The effect of sub-chronic exposure of the alkaloid extracts on inflammatory concentrations are showed in Fig. 16–18. Results showed that the concentrations of α -tumour necrosis factor ($P < 0.0001$; $F_{(16,85)} = 30.94$) and interleukin-1 β ($P < 0.0001$; $F_{(16,85)} = 119.8$) were depleted while interleukin 10 concentrations ($P < 0.0001$; $F_{(16,85)} = 94.62$) was elevated after AECS, AENT, and AECM sub-chronic exposure. However, AEDS administration elevated TNF α and IL-1 β concentrations while decreasing IL-10 concentration.

Neuromodulatory Effects of AECS, AEDS, AENT, and AECM on Redox Stress and Lactate Dehydrogenase Activity

The effect of sub-chronic exposure of the alkaloid extracts on Redox Stress is showed in Fig. 19. Results observed that AECS, AENT, and AECM sub-chronic administration inhibited reactive oxygen species production at lower doses ($P < 0.0001$; $F_{(16,85)} = 260.0$), while at 2000 mg/kg, elevated production of ROS was observed. All doses of AEDS increased reactive oxygen species production. The AECS and AENT stimulated a biphasic response ($P < 0.0001$; $F_{(16,85)} = 206.8$) in lactate dehydrogenase activity in the striatum of experimental animals when compared to the control group (Fig. 20). The administration of AECM and AEDS caused a significant ($P < 0.05$) reduction and elevation, respectively in the activities of the enzyme.

Neuromodulatory Effects of AECS, AEDS, AENT, and AECM on Gene Expression of Enzymes, Cytokines and Proteins linked to Addiction and Neurotoxicity

Figure 21–23 showed the neuromodulatory result of exposing experimental animals to AECS, AEDS, AENT, and AECM on striatal gene expressions. Result showed that AECS, AENT, and AECM administration elicited a significant ($P < 0.05$) elevation in the striatal mRNA expressions of N-methyl-D-aspartate receptor subunit 2A ($P < 0.0001$; $F_{(16,68)} = 188.0$) (Fig. 21). Also, result showed that AECS and AECM administration caused a

concentration dependent modulation in striatal and hippocampal mRNA expressions of Nuclear Factor-kappa B ($P < 0.0001$; $F_{(16, 68)} = 72.95$) and p53 ($P < 0.0001$; $F_{(16, 68)} = 210.5$) (Figs. 22 and 23).

Discussion

In understanding the neurobiology of addiction and neuropharmacological action of psychoactive plants, CPP model is established to evaluate the reinforcing abilities of a psychoactive substance-paired paradigm against a psychoactive substance-free paradigm (Möller et al., 2018; Wojcieszak et al., 2021). The CCP paradigm is principled on the theory that a textual stimulus can attain secondary appetitive ability when paired with a primary reinforcer, thus indicating abuse liability (Aguilar et al., 2009; Strekalova, 2022). This translates that AECS, AEDS, AENT and AECSM have addictive potentials via reinforcing and rewarding effects (Hur et al., 2020). Meanwhile, the significant depletion observed in the ability of alkaloid extracts-administered animals to locate hidden platform, make correct alterations and maintain movement orderliness at high doses during Y-maze and Morris water maze may indicate cognitive dysfunctions, spatial memory and learning impairments (Šlamberová et al., 2015). Inferentially, the present study showed the ability of the psychoactive plants to trigger addiction, however, at high doses, significant cognitive dysfunctions, spatial memory and learning impairments were observed, which are suggestive of neurotoxicity.

The depletion observed with the highest dose (2000 mg/kg) of the plant alkaloid extracts during CPP maybe dependent on the adverse effect observed with Y-maze and MWM tests, a validation that in order to achieve CPP, there is a need to apply memory and learning (Itzhak and Ali, 2006). This corresponds with previous studies that reported that chronic exposure to psychoactive substances at high doses will induce rapid development of tolerance and aversion, despite their potency to induce addictiveness at very low doses (Hur et al., 2020; Fasakin et al., 2022b).

The striatal dopaminergic brain pathway is a key pathway in the psychoactive, addictive and reinforcing effects of all psychoactive substances (Cunha-Oliveira et al., 2008). It is also tasked with learning and motivation; however, its overstimulation has been implicated in hyperactivity, attention deficits and attention deficit hyperactivity disorder (ADHD) (Jones and Miller, 2008; Rudin et al., 2021). Most drugs of abuse have been established to inhibit MAO activity, dopamine deaminating enzyme (Cunha-Oliveira et al., 2008; Fasakin et al., 2021). Therefore, the heightened inhibition of the enzyme activity in the present result may have resulted in elevated striatal dopaminergic concentrations. The elevated LDH activities may have also revealed the effects of the extracts on brain cells via the dopaminergic pathway as elevated LDH release have been established in heightened dopamine-concentration neurons and implicated to be suggestive of the loss of cell membrane integrity (Asanuma et al., 2020).

Glutamate (the major brain excitatory neurotransmitter) and its direct involvement in the addictive, drug seeking and dependence behaviours of drugs of abuse via the reward circuits in brain cells have been established (Danbolt et al., 2016; Yohn et al., 2019). Elevated glutamate concentrations during addiction are attributed to dopamine modulatory effects on the excitatory glutamate-releasing input and γ -aminobutyric acid-releasing output neurons (Wise and Robble, 2020). However, glutamatergic neurotransmission involving N-methyl-D-aspartate receptors (NRs) is directly involved in dependence and reinforcement in humans (Cunha-Oliveira et al., 2008). A functional NMDA receptor (NR) entails a minimum of NR1 subunit that consist a glycine binding site and the other consisting a glutamate binding site each (Ehsanifar et al., 2019). The inhibition of glutamate

dehydrogenase activity and the activation of NR2A may be indicative that the stimulation of glutamatergic NMDA receptors is involved in facilitating the addictive-properties of the psychoactive plants (Lobo and Summavielle, 2016; Josiah et al., 2021; Fasakin et al., 2022a). Overstimulation of the glutamatergic NMDA receptors has been implicated to result in mitochondria impairments and excitotoxicity (Myslivecek, 2021; Josiah et al., 2022), which is suggestive of the neurotoxicological potential of AEDS at high dose.

The cholinergic neurotransmission system has been implicated to induce addiction via its involvement in mesolimbic dopamine activities (Yohn et al., 2019). Likewise, the elevation of striatal dopamine concentrations during addiction has been observed to be dependent on muscarinic and nicotinic acetylcholine receptors (mAChRs) activation (Lester et al., 2010; Faro et al., 2019), an indication that dopaminergic and cholinergic systems are intertwined during addiction. The involvement of cholinergic systems in glutamatergic terminals have also been established during the dependence and reinforcing-producing actions of nicotine (Lester et al., 2010; Myslivecek, 2021). The inhibitory effects on acetylcholinesterase may be suggestive of the possible addictive mechanism of the plants, as acetylcholinesterase is the principal factor underlining upregulated cholinergic neurotransmission and homeostatic acetylcholine concentrations (Fasakin et al., 2022a; Ademosun et al. 2023). Extensive acetylcholinesterase inhibition results in excess synaptic acetylcholine concentrations, altered postsynaptic cell function, overstimulated cholinergic receptors and cholinergic toxicity (Pope et al., 2005). AEDS administration elicited the highest inhibitory effects on acetylcholinesterase despite posing a non-significant effect on dopamine release, a probable validator that *D. stramonium*-induced acetylcholine release may be dependent on interactions between acetylcholine and other several catecholamines rather than solely dopamine (Ferrucci et al., 2019).

The high oxidizability of dopamine alongside high concentrations of dopamine observed in this study may have resulted in the aggravated ROS concentrations observed in the study. The inappropriate sequestering of dopamine into vesicles results in high cytosolic dopamine concentrations that has been implicated to result in elevated quinones, ROS and other toxic intermediates production via autoxidation, enzymatic reactions and metabolisms (Carvalho et al. 2012; Goldstein et al. 2012; Masoud et al. 2015). Quinones undergo redox cycle to form semiquinone radicals that result in superoxide radicals' generation, which may then react with the elevated nitric oxide levels observed in the present study to potentiate neurotoxin peroxynitrite production which have been implicated in cellular proteins and DNA damages via interactions with thiol groups (Rudin et al. 2021). Additionally, dopamine autoxidation results in hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\cdot -}$) production, with the later having ability to react with elevated nitric oxide concentrations to form highly neurotoxic peroxynitrite (Cunha-Oliveira et al., 2008).

The induced-oxidative stress can trigger inflammatory responses characterized by heightened upregulation of proinflammatory mediators (such as TNF- α , NF- κ B, IL-1 β) and inhibition of anti-inflammatory mediators (such as IL-10) to stimulate apoptotic pathway (Fasakin et al., 2022a; Josaih et al., 2022). Neuroimmune response to addiction is characterized by the stimulation of microglia that leads to elevated proinflammatory cytokines production that interacts with neurotransmitters in related brain circuits (Flores-López et al. 2021; Gipson et al., 2021). In fact, brain pro-inflammatory neuroimmune signalling have been established to regulate GABA and AMPA receptor trafficking during addiction (Gipson et al., 2021). The oxidative stress stimulated-inflammatory responses can then initiate programmed-apoptosis via the activation of NF κ B-dependent p53 signalling pathway and thereby, upregulated p53 gene expression which have been established to trigger loss and death of striatal dopamine-producing cells (Yan et al. 2014; Josiah et al., 2022). Elevated IL-1 β and TNF- α concentrations can

also directly induce neuronal apoptosis by stimulating adaptive and innate immune cells, and excessive glial hyper-activation (Tjalkens et al., 2017). This cascade coupled with the stimulation of NFκB and p53 gene expression of experimental rats' striatum at the high doses of the alkaloid extracts may be indicative of addiction-induced neurodegeneration and cell death.

Purinergic neurotransmitters have also been implicated in the aetiology of addiction and neurotoxicity (Rozisky et al., 2010; Wu and Li, 2020). ATP and ADP are hydrolysed by E-NTPDases while AMP are hydrolysed by ecto-5'-nucleotidase to adenosine. Dopamine has also been postulated to act on appropriate dopamine receptors to elevate adenosine concentrations (Kombian et al., 2003). Resulting adenosine can then act via specific G-protein-coupled P1 purinoceptors, A_{2A}-D₂ receptor-receptor and A_{2A}R interactions in brain's dorsal striatum to mediate addiction and habitual substance seeking behaviours (Pintsuk et al., 2016; Burnstock, 2017). However, stimulation of the striatal A_{2A}R have been implicated to mediate fear, and elevate glutamate induced-excitotoxicity and interleukin-1β concentrations; indicators of neuroinflammation, depression and anxiety (Mayhew et al., 2018; Fasakin et al., 2022a). Observed E-NTPDase activity in this study is suggestive of elevated ATP hydrolysis, an indication of depleted bioenergetics (Leong et al. 2020). Also, the elevation of E-NTPDase activity resulting in reduced cAMP formation, information loss and cognitive dysfunction have also been established (Ademosun et al., 2022). Contra-wise, the inhibition of ecto-5'-nucleotidase and E-NTPDase activities by AEDS have been postulated to induce neurotoxicity via excessive inhibition of purinergic neurotransmission systems, a cascade that has proven more neurotoxic than that experienced with overstimulation of the adrenergic neurotransmission system (Fasakin et al., 2021).

Conclusion

The result of this study showed a strong linked between the addictiveness and neurotoxicological potentials of *Cannabis sativa*, male *Carica papaya* and *Nicotiana tabacum*, and *Datura stramonium*. However, the neurotoxicological potentials of the psychoactive plants were not directly proportional to their addictiveness as the psychoactive plants' alkaloids ranked AECS > AENT > AEDS > AECM in addictiveness but ranked AEDS > AENT > AECS > AECM for toxicological potentials. Notably, the toxicological effects of the psychoactive plants' alkaloids are mostly expressed at high doses. However, further studies are recommended to device how the knowledge of discussed brain systems' involvement can be employed during treatment and management of addiction and neurotoxicity.

Declarations

Conflicts of interest

All authors declare no conflict of interest

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Informed Consent

All the authors contributed substantially to the work, participated in the writing and have seen and approved the submitted version.

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Ethical Approval

The care and use of Laboratory Animals were approved by the Federal University of Technology Akure ethical committee, which was followed strictly and in compliance with the National Institute of Health guidelines. Ethical approval was obtained from the Centre for Research and Development (CERAD), Federal University of Technology, Akure with the number FUTA/ETH/2020/016.

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Figures

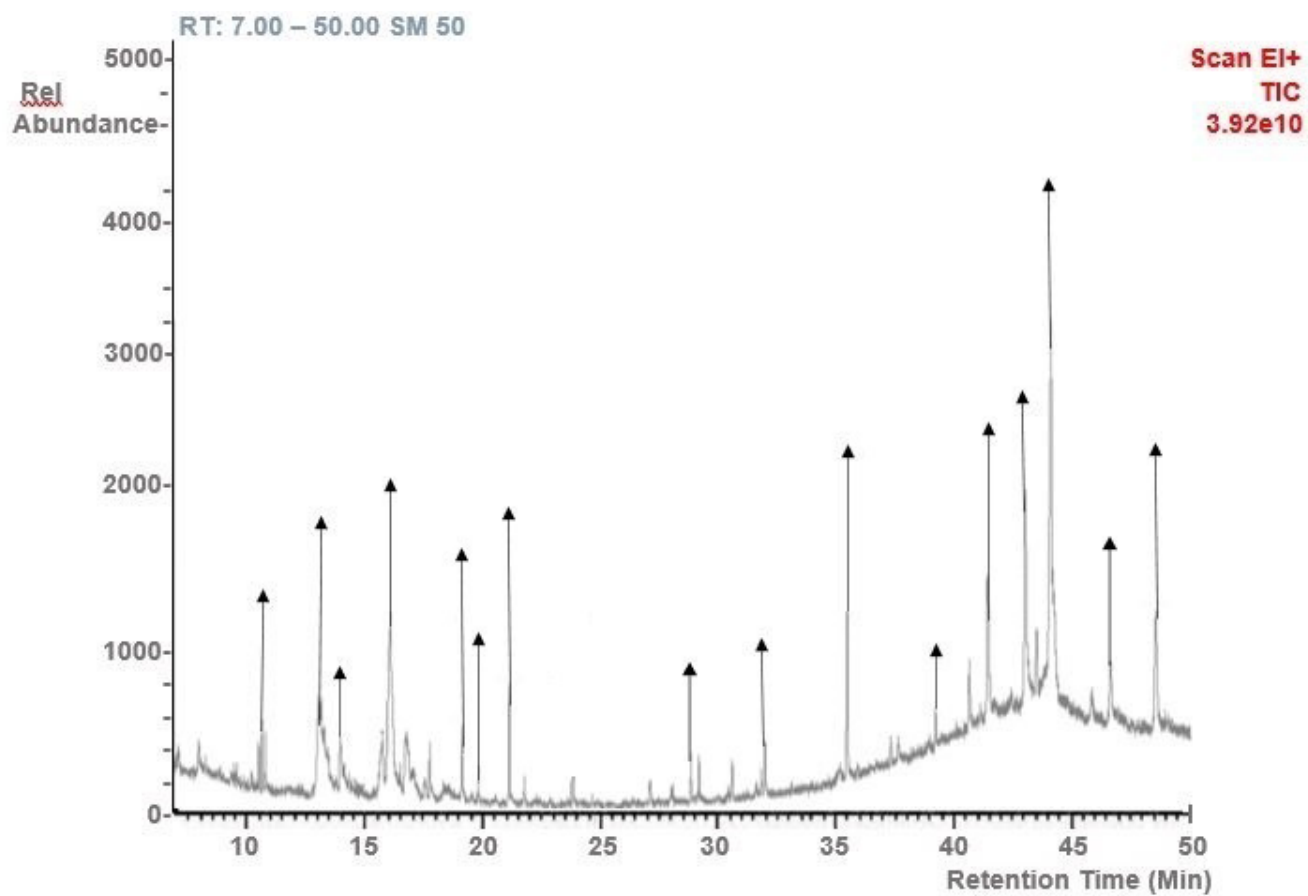


Figure 1

GC-MS characterization of AECS.

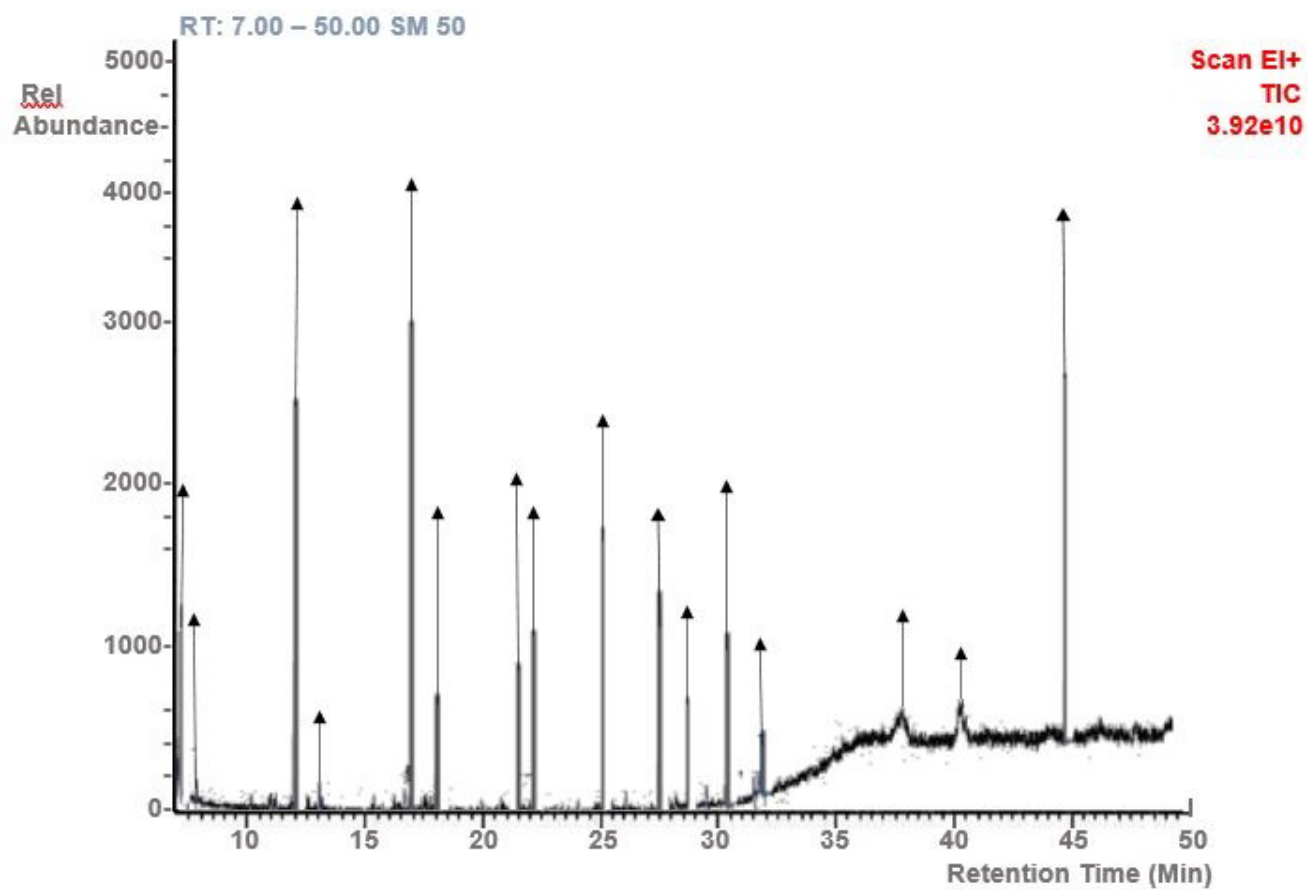


Figure 2

GC-MS characterization of AEDS.

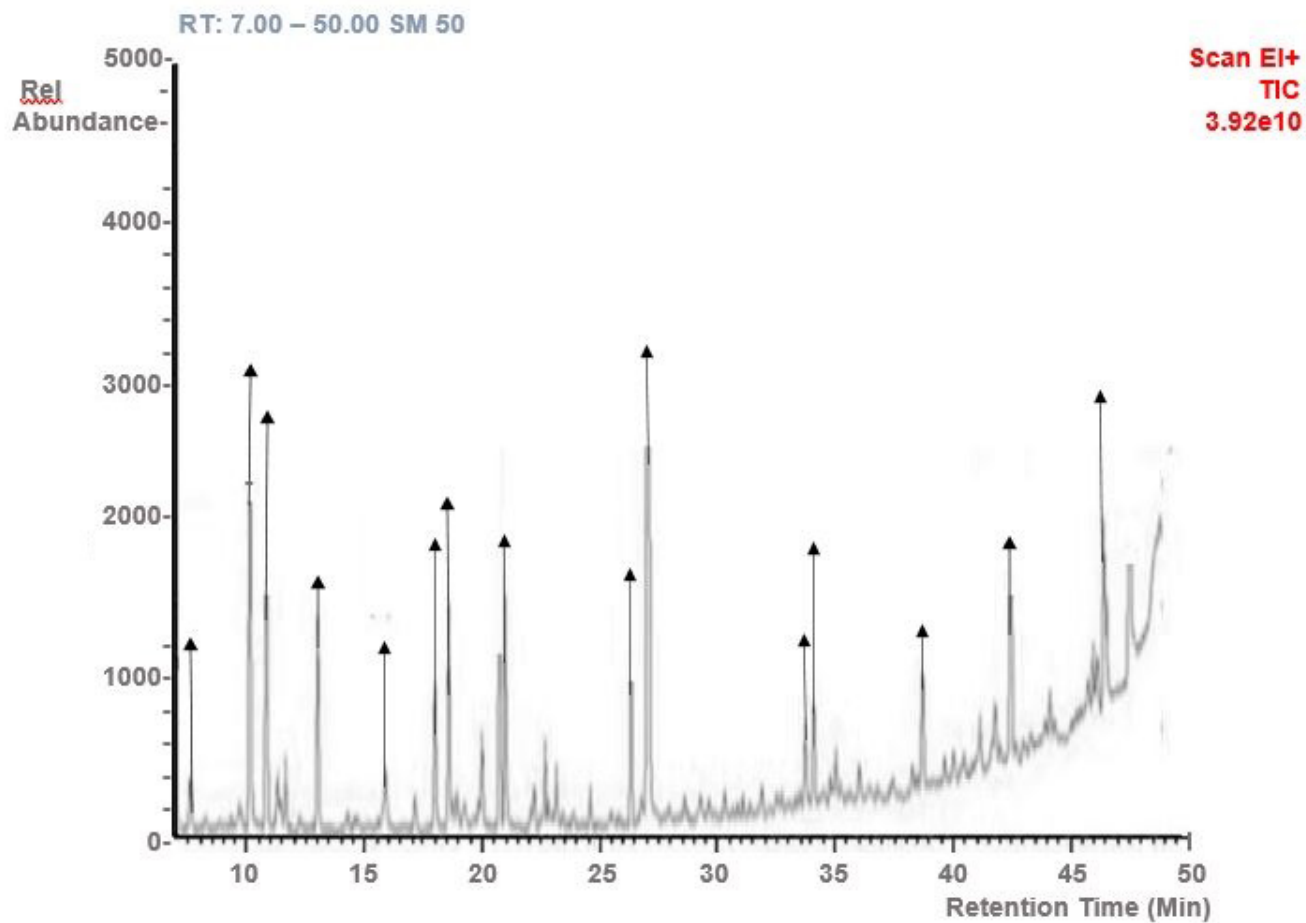


Figure 3

GC-MS characterization of AENT.

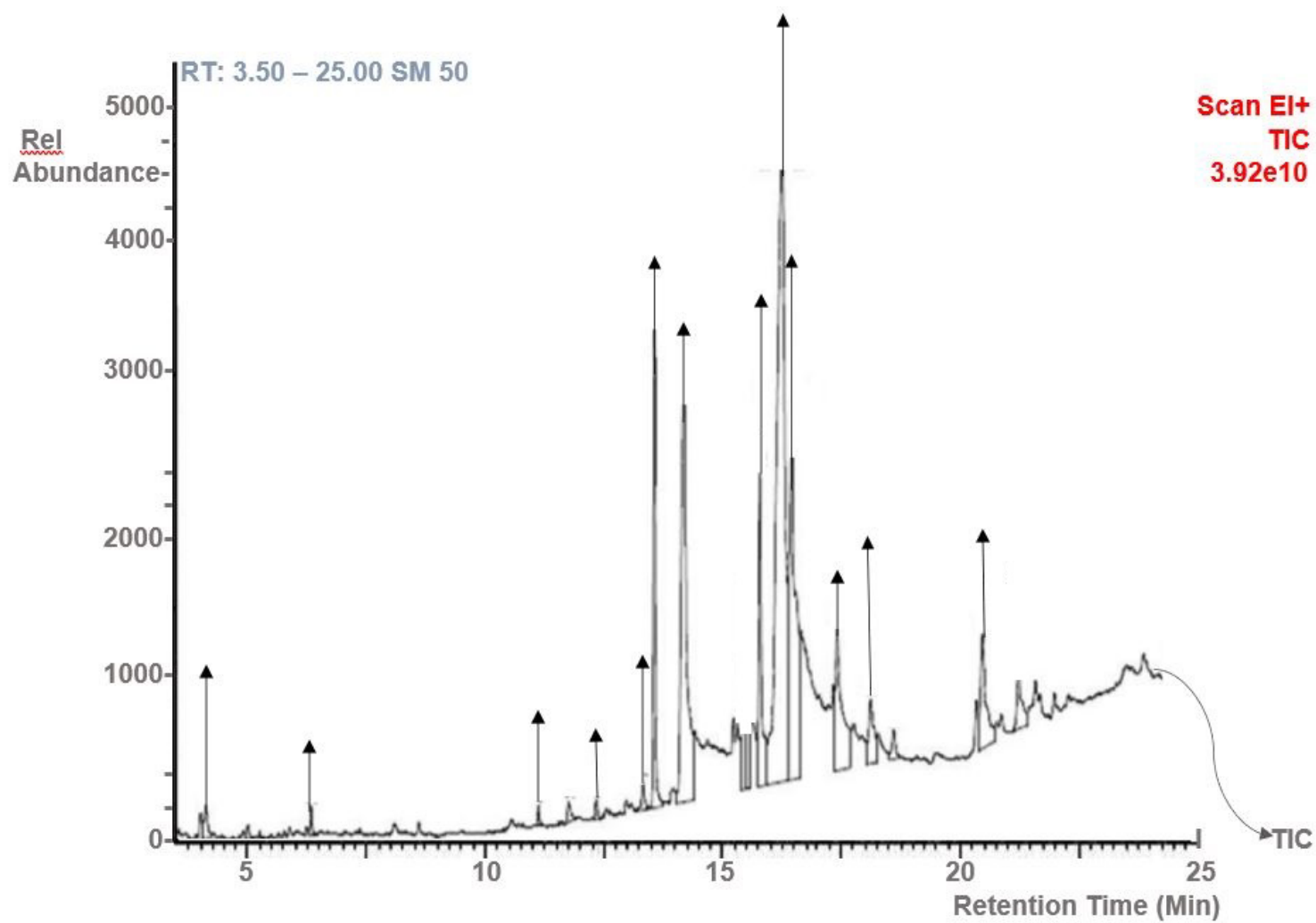


Figure 4

GC-MS characterization of AECM.

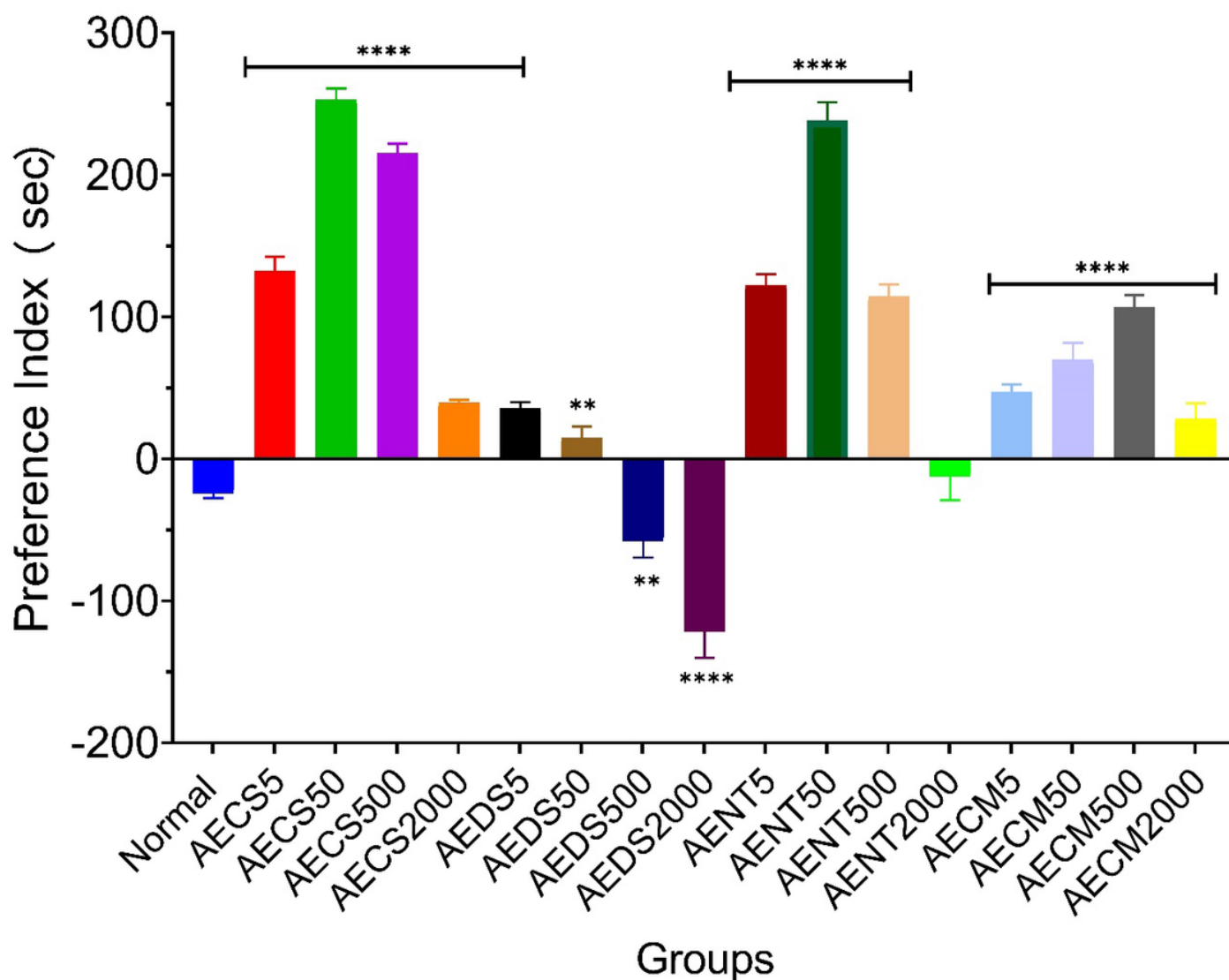


Figure 5

Effects of CSAE, DSAE, NTAE, and CMAE on preference index during conditioned place preference (CPP).

Results are expressed as mean $\pm$ SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group. AECS5 – orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; AECS50 – orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; AECS500 – orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; AECS2000 – orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; AEDS5 – orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; AEDS50 – orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; AEDS500 – orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; AEDS2000 – orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; AENT5 – orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; AENT50 – orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; AENT500 – orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; AENT2000 – orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; AECSM5 – orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; AECSM50 – orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; AECSM500 – orally administered

alkaloid extracts of *C. papaya* at 500 mg/kg bwt; AECM2000 – orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

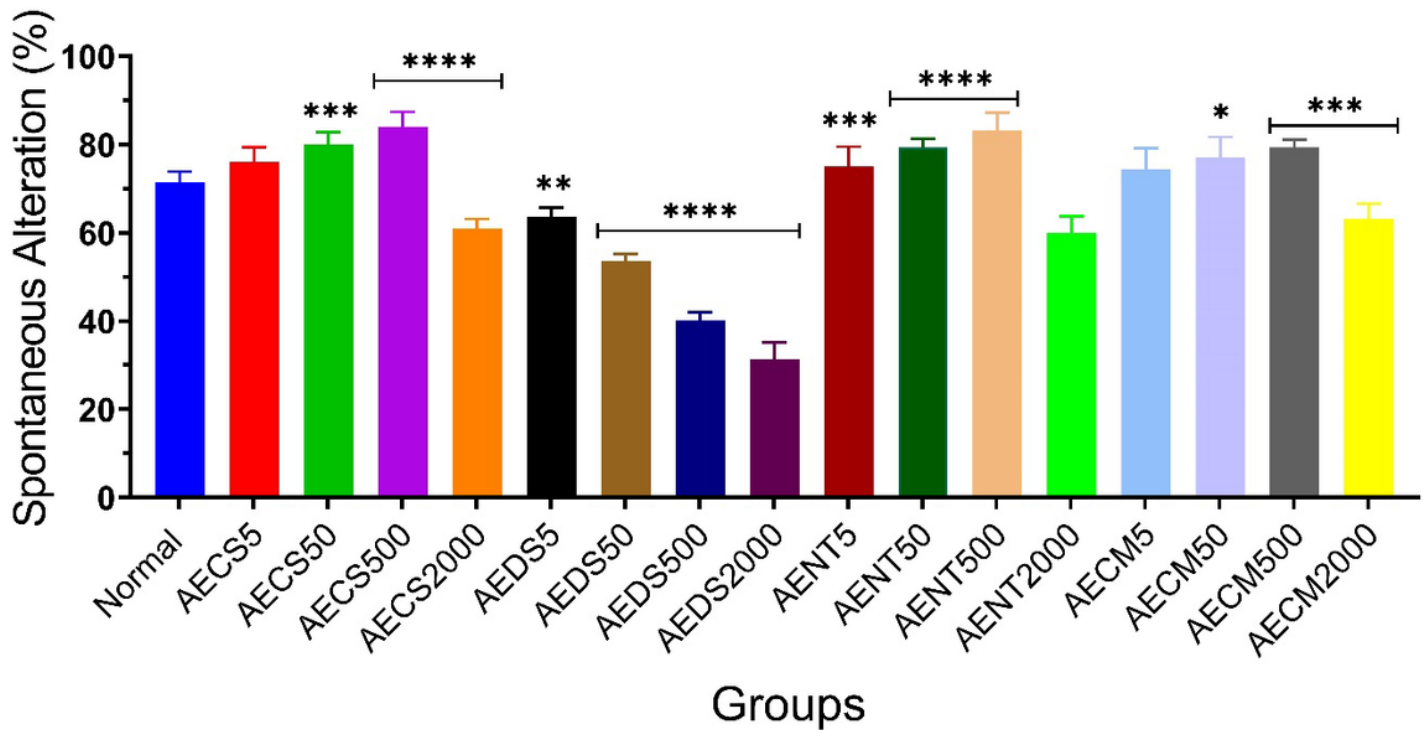


Figure 6

Effects of CSAE, DSAE, NTAE, and CMAE on spontaneous alternation using Y-Maze.

Results are expressed as mean $\pm$ SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group. AECS5 – orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; AECS50 – orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; AECS500 – orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; AECS2000 – orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; AEDS5 – orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; AEDS50 – orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; AEDS500 – orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; AEDS2000 – orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; AENT5 – orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; AENT50 – orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; AENT500 – orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; AENT2000 – orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; AECM5 – orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; AECM50 – orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; AECM500 – orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; AECM2000 – orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

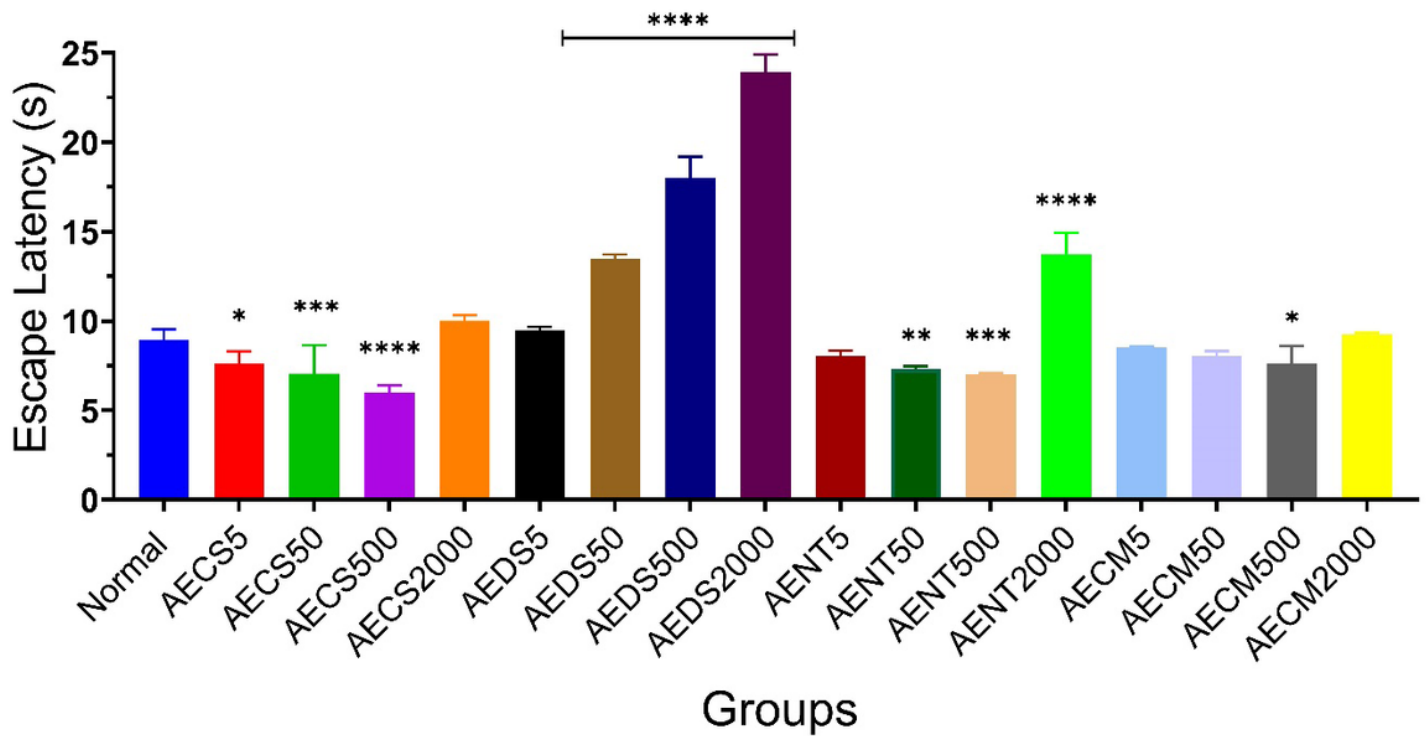


Figure 7

Effects of CSAE, DSAE, NTAE, and CMAEon escape latency using Morris Water Maze test.

Results are expressed as mean $\pm$ SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group. AECS5 – orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; AECS50 – orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; AECS500 – orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; AECS2000 – orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; AEDS5–orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; AEDS50 – orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; AEDS500 – orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; AEDS2000 – orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; AENT5 – orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; AENT50 – orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; AENT500 – orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; AENT2000 – orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; AECM5 – orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; AECM50 – orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; AECM500 – orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; AECM2000 – orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

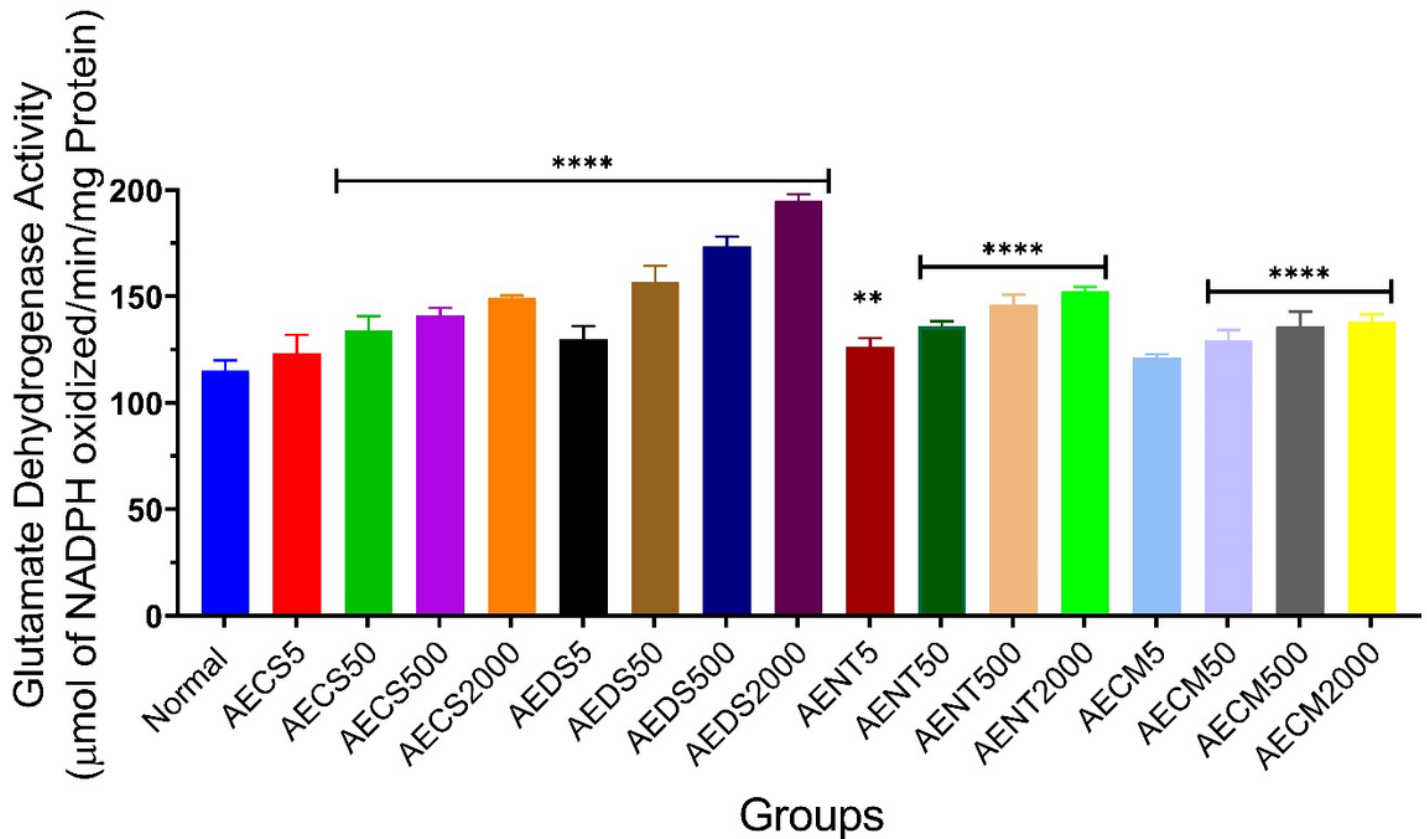


Figure 8

Effects of CSAE, DSAE, NTAE, and CMAE on Glutamate dehydrogenase Activities.

Results are expressed as mean $\pm$ SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group. AECS5 – orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; AECS50 – orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; AECS500 – orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; AECS2000 – orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; AEDS5 – orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; AEDS50 – orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; AEDS500 – orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; AEDS2000 – orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; AENT5 – orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; AENT50 – orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; AENT500 – orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; AENT2000 – orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; AECM5 – orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; AECM50 – orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; AECM500 – orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; AECM2000 – orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

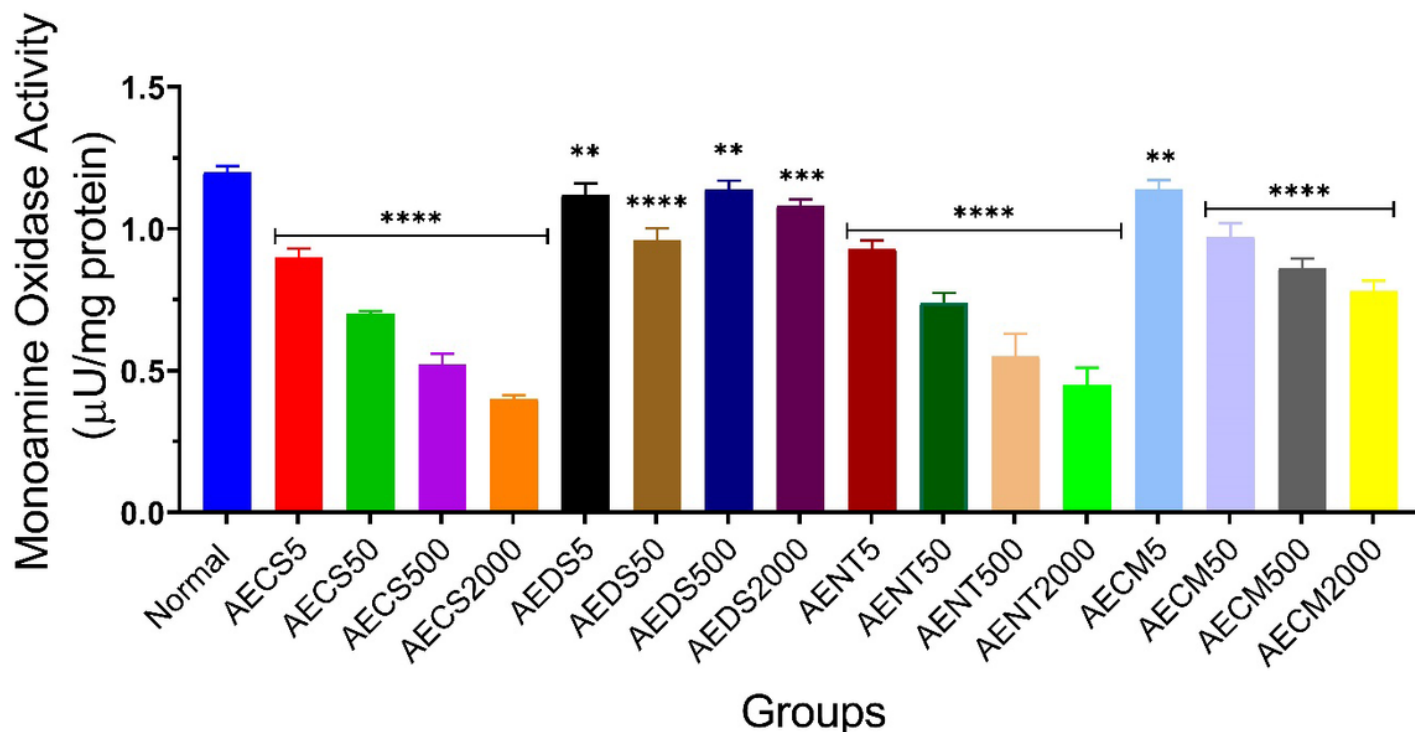


Figure 9

Effects of CSAE, DSAE, NTAE, and CMAE on Monoamine Oxidase Activities.

Results are expressed as mean $\pm$ SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group. AECS5 – orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; AECS50 – orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; AECS500 – orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; AECS2000 – orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; AEDS5 – orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; AEDS50 – orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; AEDS500 – orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; AEDS2000 – orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; AENT5 – orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; AENT50 – orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; AENT500 – orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; AENT2000 – orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; AECM5 – orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; AECM50 – orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; AECM500 – orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; AECM2000 – orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

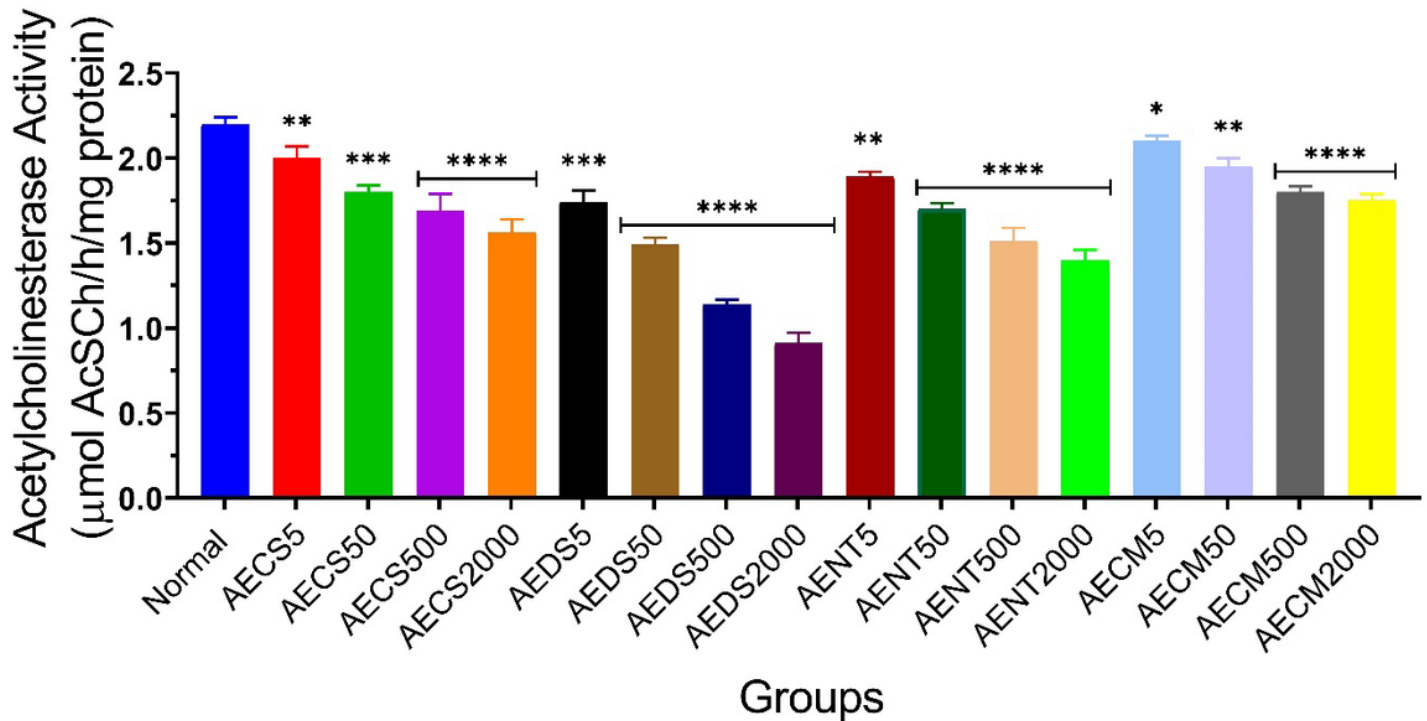


Figure 10

Effects of CSAE, DSAE, NTAE, and CMAE on Acetylcholinesterase Activities.

Results are expressed as mean $\pm$ SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group. AECS5 – orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; AECS50 – orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; AECS500 – orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; AECS2000 – orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; AEDS5 – orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; AEDS50 – orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; AEDS500 – orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; AEDS2000 – orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; AENT5 – orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; AENT50 – orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; AENT500 – orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; AENT2000 – orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; AECM5 – orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; AECM50 – orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; AECM500 – orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; AECM2000 – orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

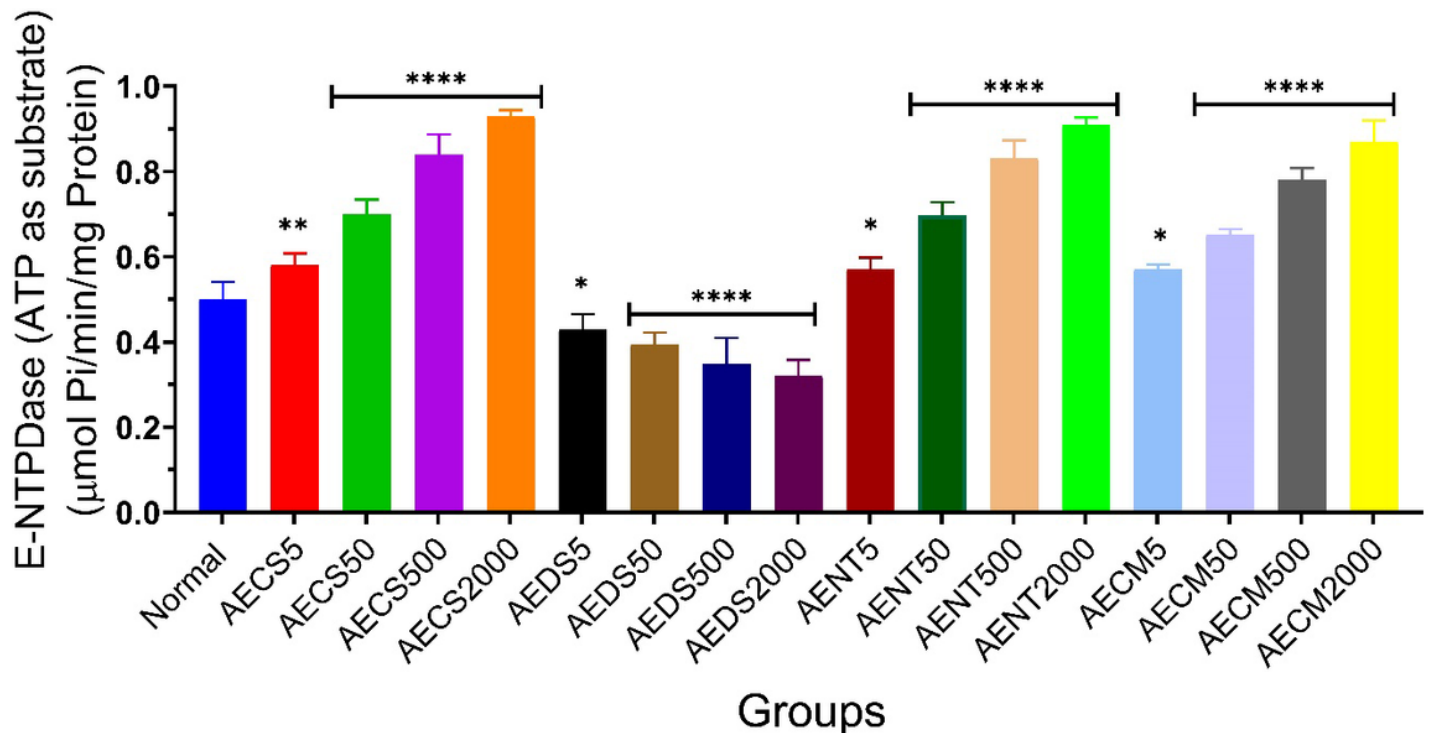


Figure 11

Effects of CSAE, DSAE, NTAE, and CMAE on E-NTPDase (ATP) Activities.

Results are expressed as mean $\pm$ SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group. AECS5 – orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; AECS50 – orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; AECS500 – orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; AECS2000 – orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; AEDS5 – orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; AEDS50 – orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; AEDS500 – orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; AEDS2000 – orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; AENT5 – orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; AENT50 – orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; AENT500 – orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; AENT2000 – orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; AECM5 – orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; AECM50 – orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; AECM500 – orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; AECM2000 – orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

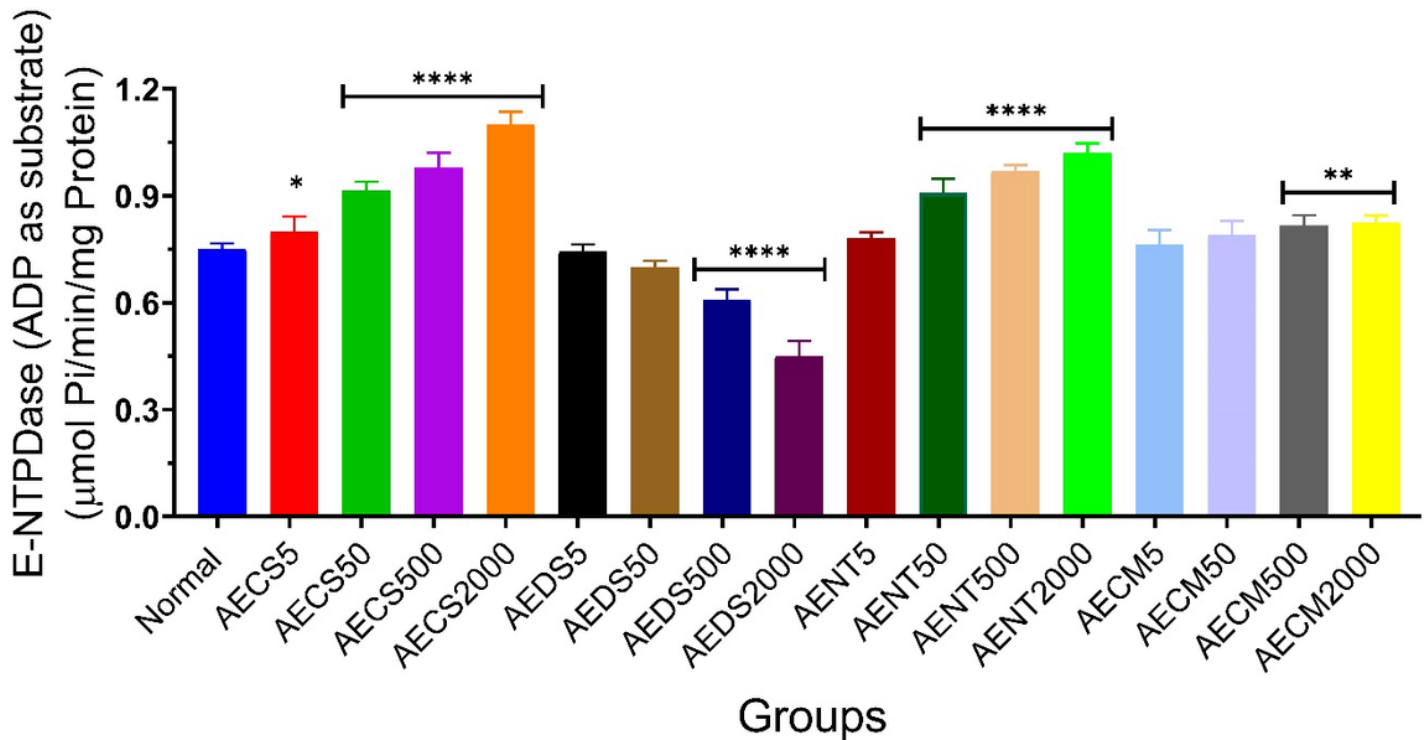


Figure 12

Effects of CSAE, DSAE, NTAE, and CMAE on E-NTPDase (ADP) Activities.

Results are expressed as mean $\pm$ SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group. AECS5 – orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; AECS50 – orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; AECS500 – orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; AECS2000 – orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; AEDS5 – orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; AEDS50 – orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; AEDS500 – orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; AEDS2000 – orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; AENT5 – orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; AENT50 – orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; AENT500 – orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; AENT2000 – orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; AECM5 – orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; AECM50 – orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; AECM500 – orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; AECM2000 – orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

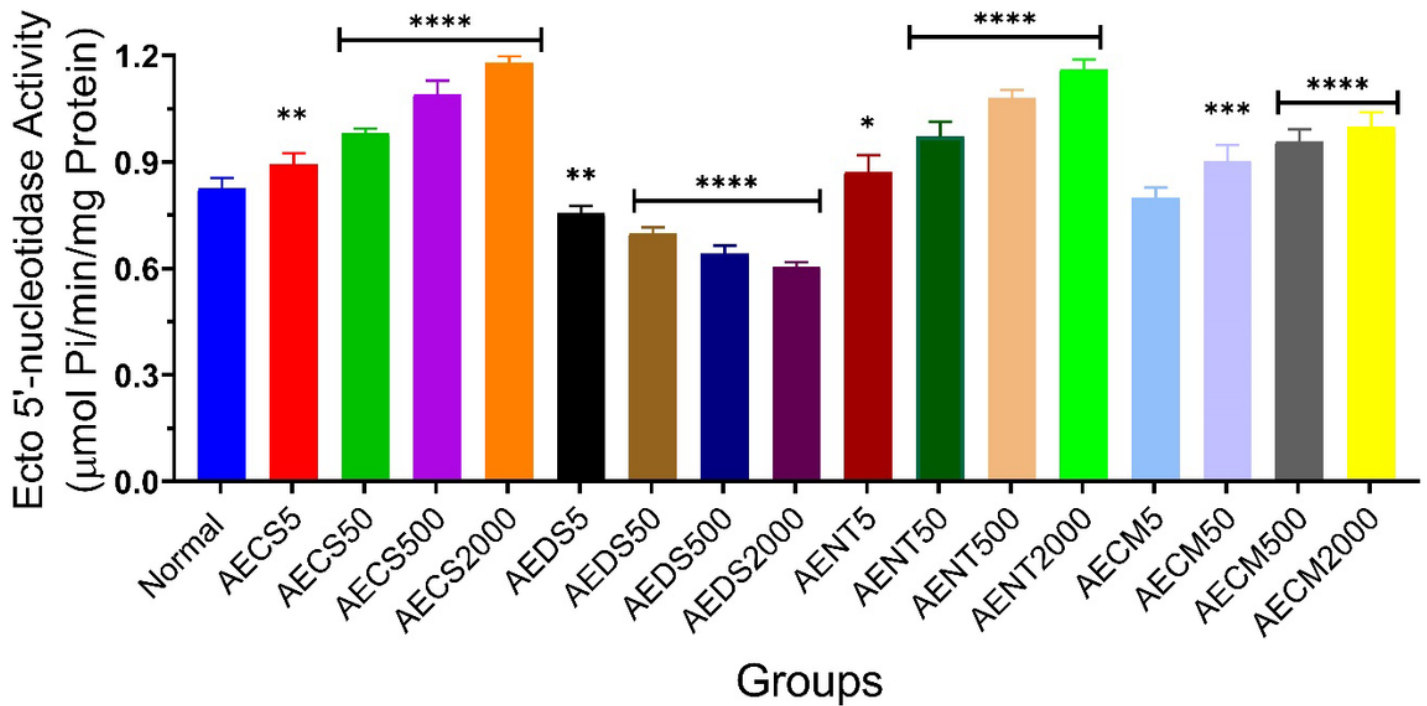


Figure 13

Effects of CSAE, DSAE, NTAE, and CMAE on ecto-5'-nucleotidase Activities.

Results are expressed as mean $\pm$ SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group. AECS5 – orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; AECS50 – orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; AECS500 – orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; AECS2000 – orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; AEDS5 – orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; AEDS50 – orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; AEDS500 – orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; AEDS2000 – orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; AENT5 – orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; AENT50 – orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; AENT500 – orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; AENT2000 – orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; AECM5 – orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; AECM50 – orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; AECM500 – orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; AECM2000 – orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

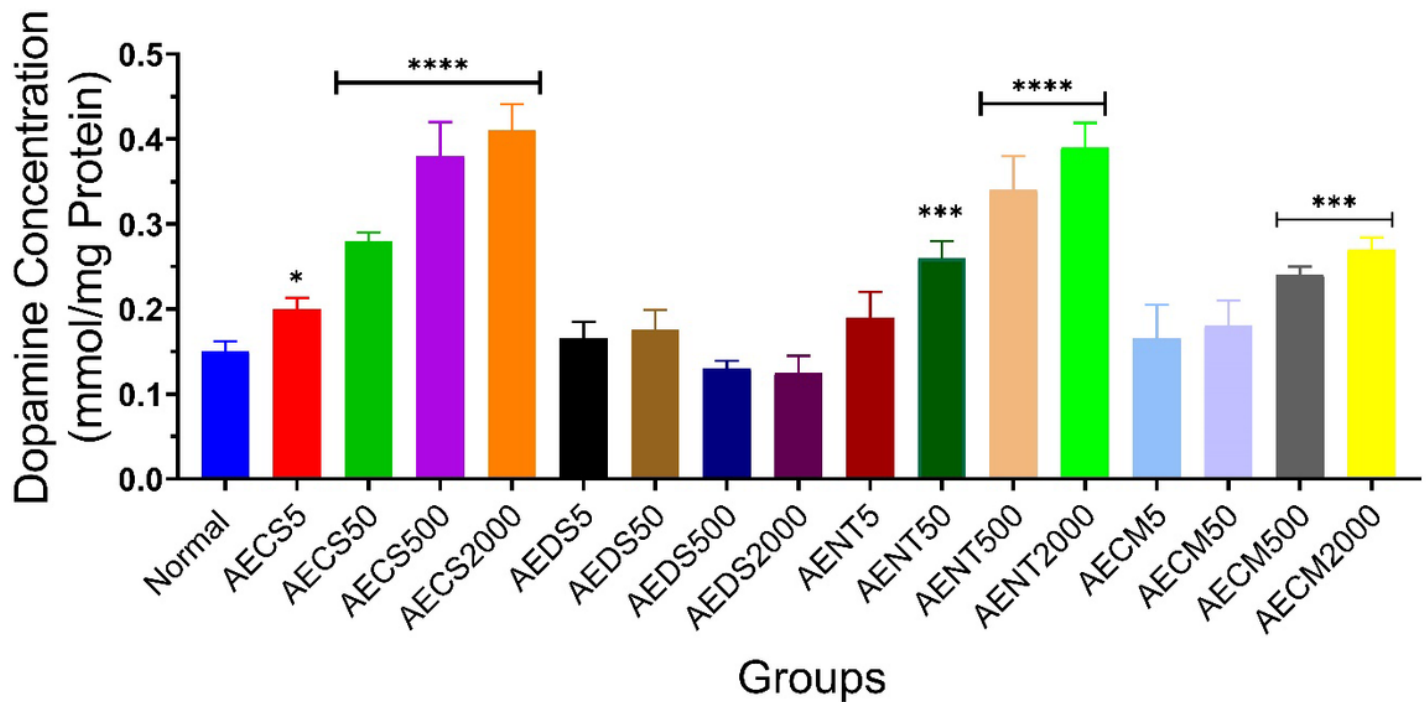


Figure 14

Effects of CSAE, DSAE, NTAE, and CMAE on Dopamine Concentration.

Results are expressed as mean $\pm$ SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group. AECS5 – orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; AECS50 – orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; AECS500 – orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; AECS2000 – orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; AEDS5 – orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; AEDS50 – orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; AEDS500 – orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; AEDS2000 – orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; AENT5 – orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; AENT50 – orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; AENT500 – orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; AENT2000 – orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; AECSM5 – orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; AECSM50 – orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; AECSM500 – orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; AECSM2000 – orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

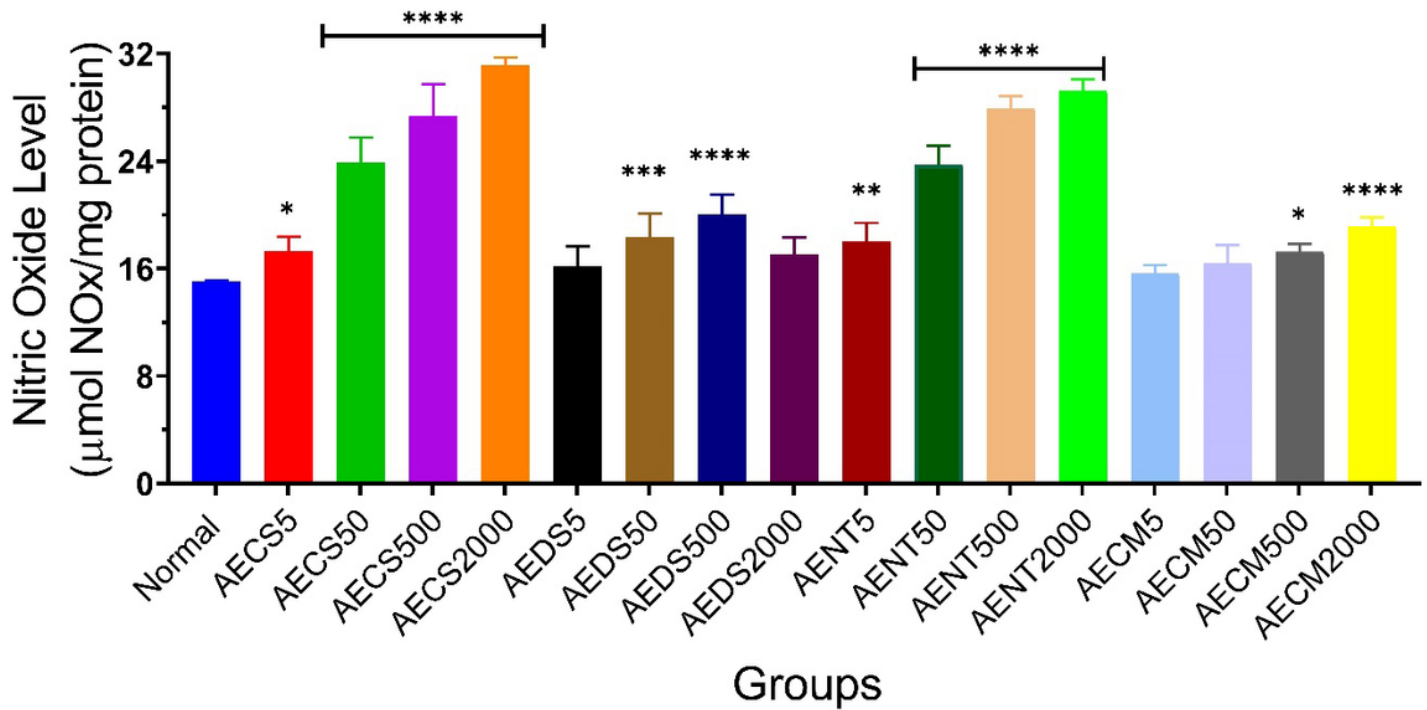


Figure 15

Effects of CSAE, DSAE, NTAE, and CMAE on Nitric oxide Concentration.

Results are expressed as mean $\pm$ SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group. AECS5 – orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; AECS50 – orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; AECS500 – orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; AECS2000 – orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; AEDS5 – orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; AEDS50 – orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; AEDS500 – orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; AEDS2000 – orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; AENT5 – orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; AENT50 – orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; AENT500 – orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; AENT2000 – orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; AECM5 – orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; AECM50 – orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; AECM500 – orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; AECM2000 – orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

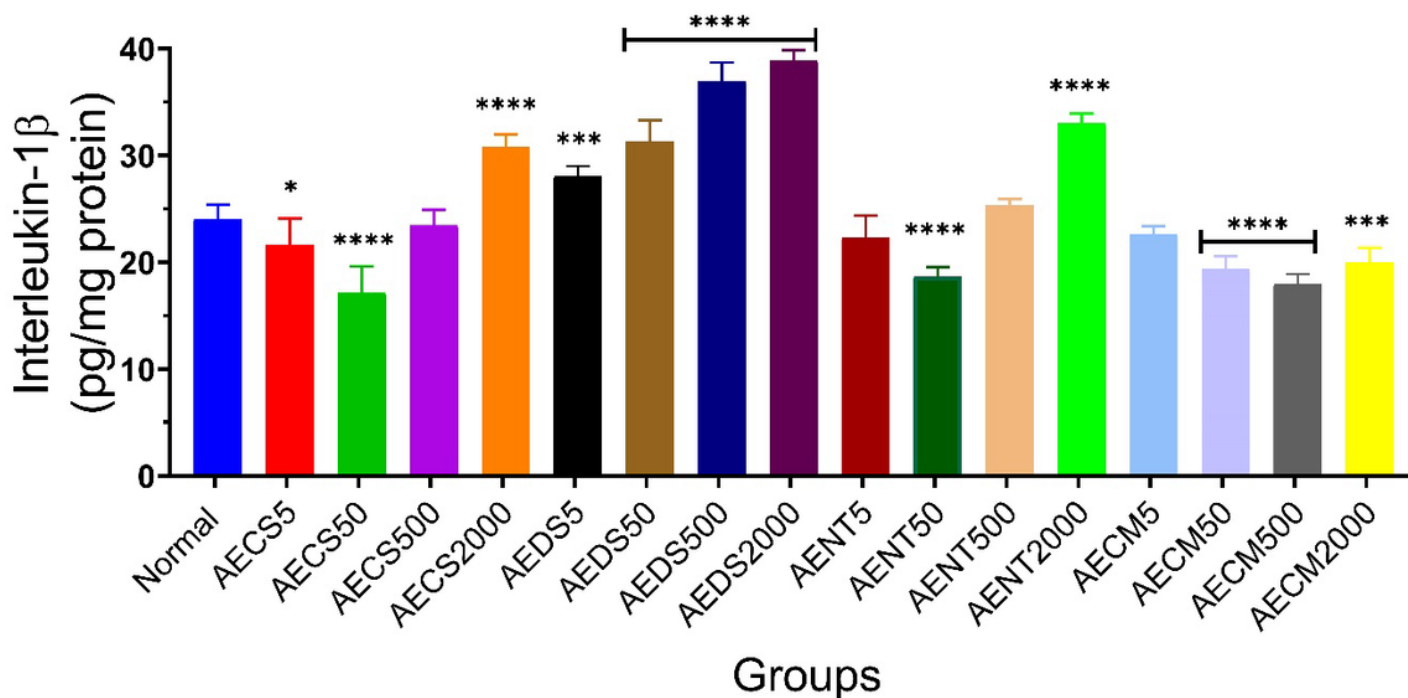


Figure 17

Effects of CSAE, DSAE, NTAE, and CMAE on interleukin-1 β Concentration.

Results are expressed as mean $\pm$ SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group. AECS5 – orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; AECS50 – orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; AECS500 – orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; AECS2000 – orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; AEDS5 – orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; AEDS50 – orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; AEDS500 – orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; AEDS2000 – orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; AENT5 – orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; AENT50 – orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; AENT500 – orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; AENT2000 – orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; AECM5 – orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; AECM50 – orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; AECM500 – orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; AECM2000 – orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

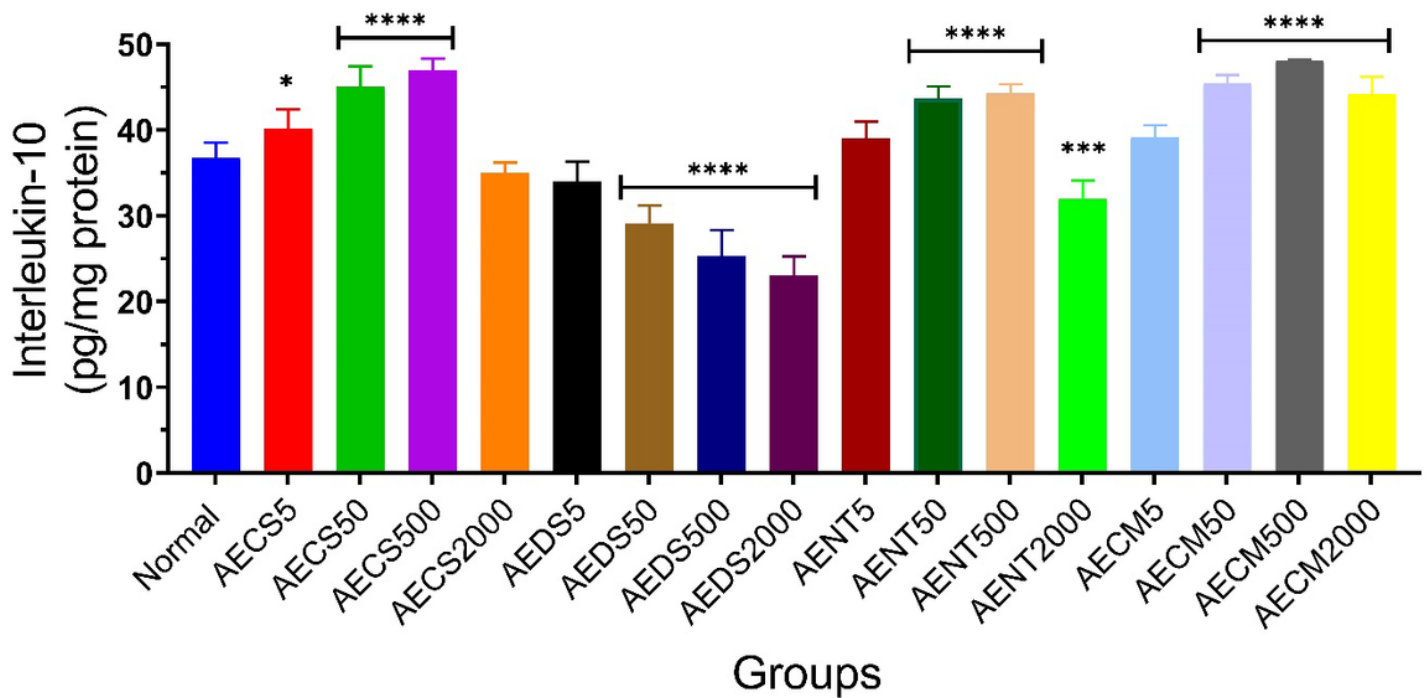


Figure 18

Effects of CSAE, DSAE, NTAE, and CMAE on interleukin-10 Concentration.

Results are expressed as mean $\pm$ SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group. AECS5 – orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; AECS50 – orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; AECS500 – orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; AECS2000 – orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; AEDS5 – orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; AEDS50 – orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; AEDS500 – orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; AEDS2000 – orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; AENT5 – orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; AENT50 – orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; AENT500 – orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; AENT2000 – orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; AECM5 – orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; AECM50 – orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; AECM500 – orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; AECM2000 – orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

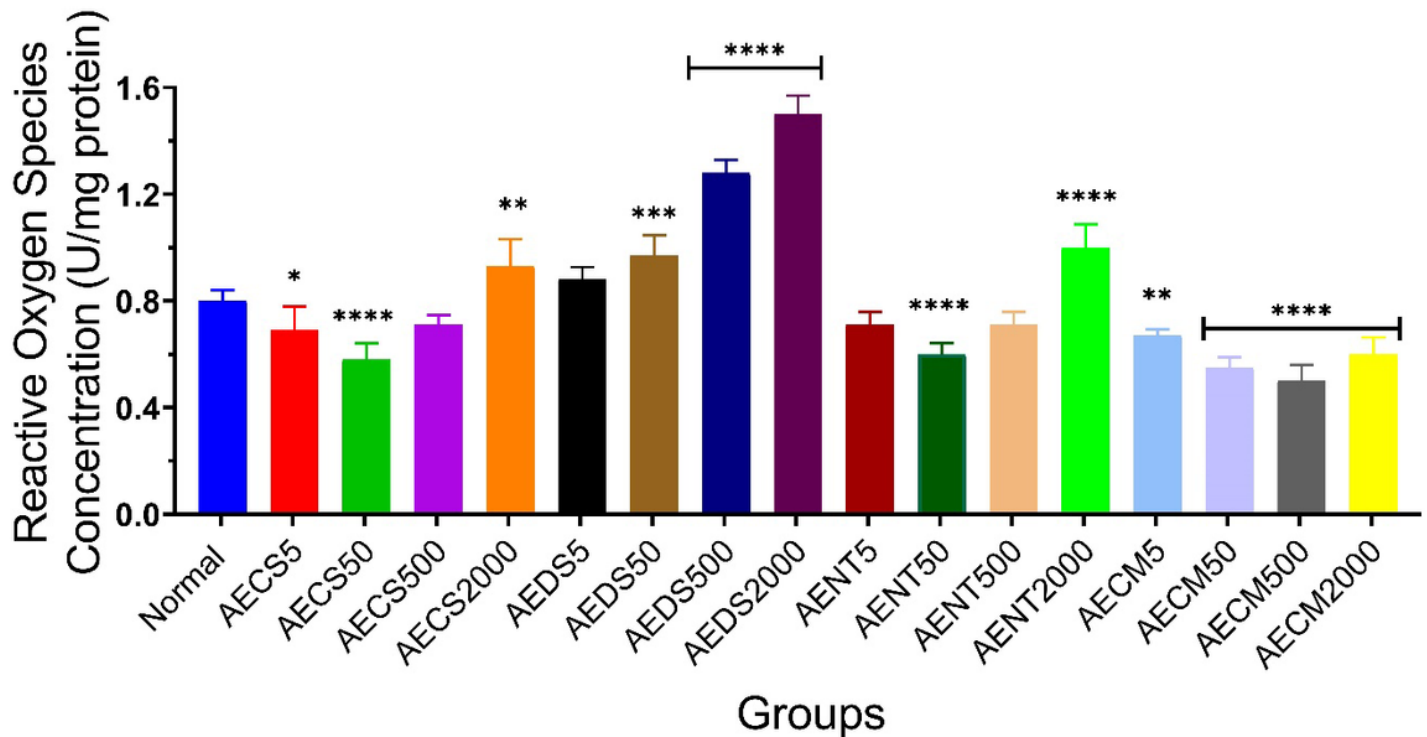


Figure 19

Effects of CSAE, DSAE, NTAE, and CMAE on Reactive oxygen species production.

Results are expressed as mean $\pm$ SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group. AECS5 – orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; AECS50 – orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; AECS500 – orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; AECS2000 – orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; AEDS5 – orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; AEDS50 – orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; AEDS500 – orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; AEDS2000 – orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; AENT5 – orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; AENT50 – orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; AENT500 – orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; AENT2000 – orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; AECM5 – orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; AECM50 – orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; AECM500 – orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; AECM2000 – orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

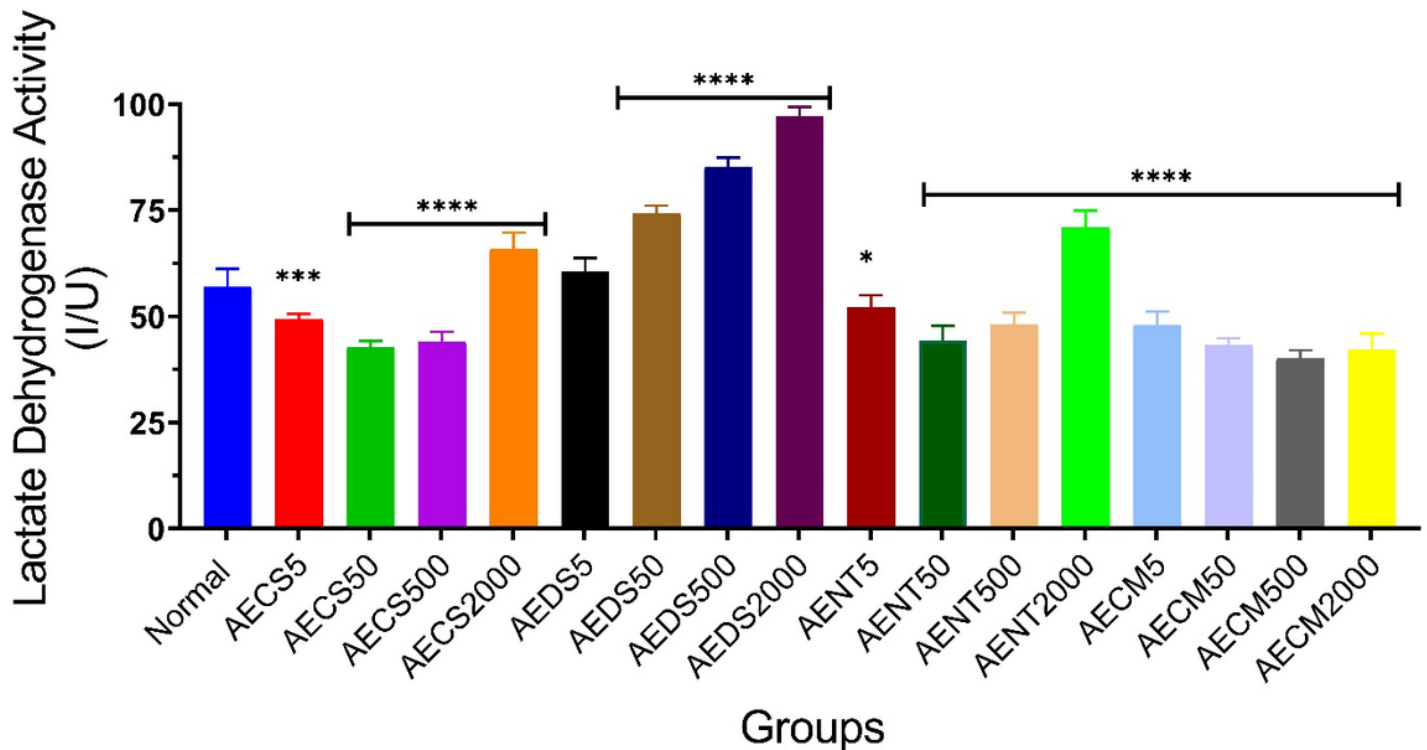


Figure 20

Effects of CSAE, DSAE, NTAE, and CMAE on Lactate dehydrogenase Activities.

Results are expressed as mean $\pm$ SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group. AECS5 – orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; AECS50 – orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; AECS500 – orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; AECS2000 – orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; AEDS5 – orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; AEDS50 – orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; AEDS500 – orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; AEDS2000 – orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; AENT5 – orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; AENT50 – orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; AENT500 – orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; AENT2000 – orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; AECS5 – orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; AECS50 – orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; AECS500 – orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; AECS2000 – orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

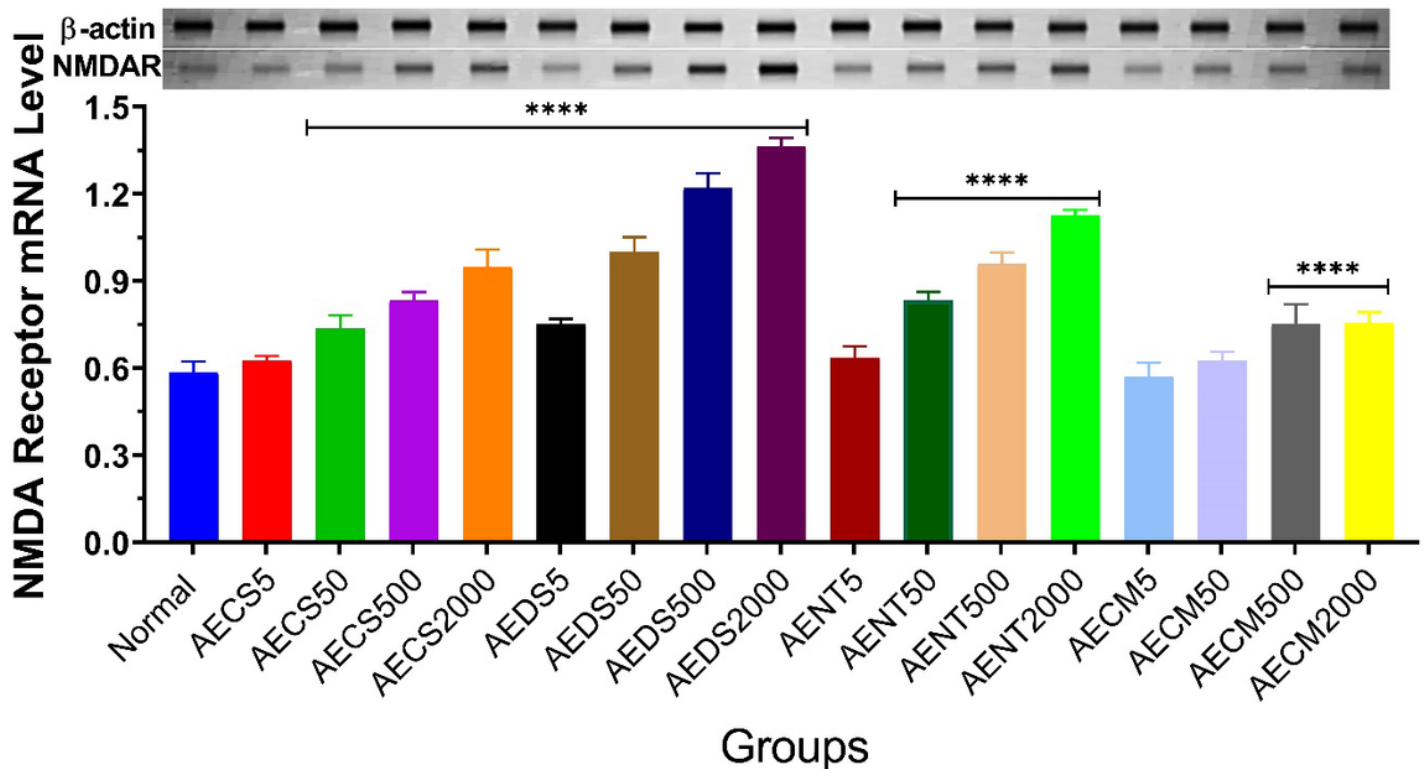


Figure 21

Effects of CSAE, DSAE, NTAE, and CMAE on N-methyl-D-aspartate receptor subunit 2A mRNA expression.

Results are expressed as mean $\pm$ SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group. AECS5 – orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; AECS50 – orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; AECS500 – orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; AECS2000 – orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; AEDS5 – orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; AEDS50 – orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; AEDS500 – orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; AEDS2000 – orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; AENT5 – orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; AENT50 – orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; AENT500 – orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; AENT2000 – orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; AECM5 – orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; AECM50 – orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; AECM500 – orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; AECM2000 – orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

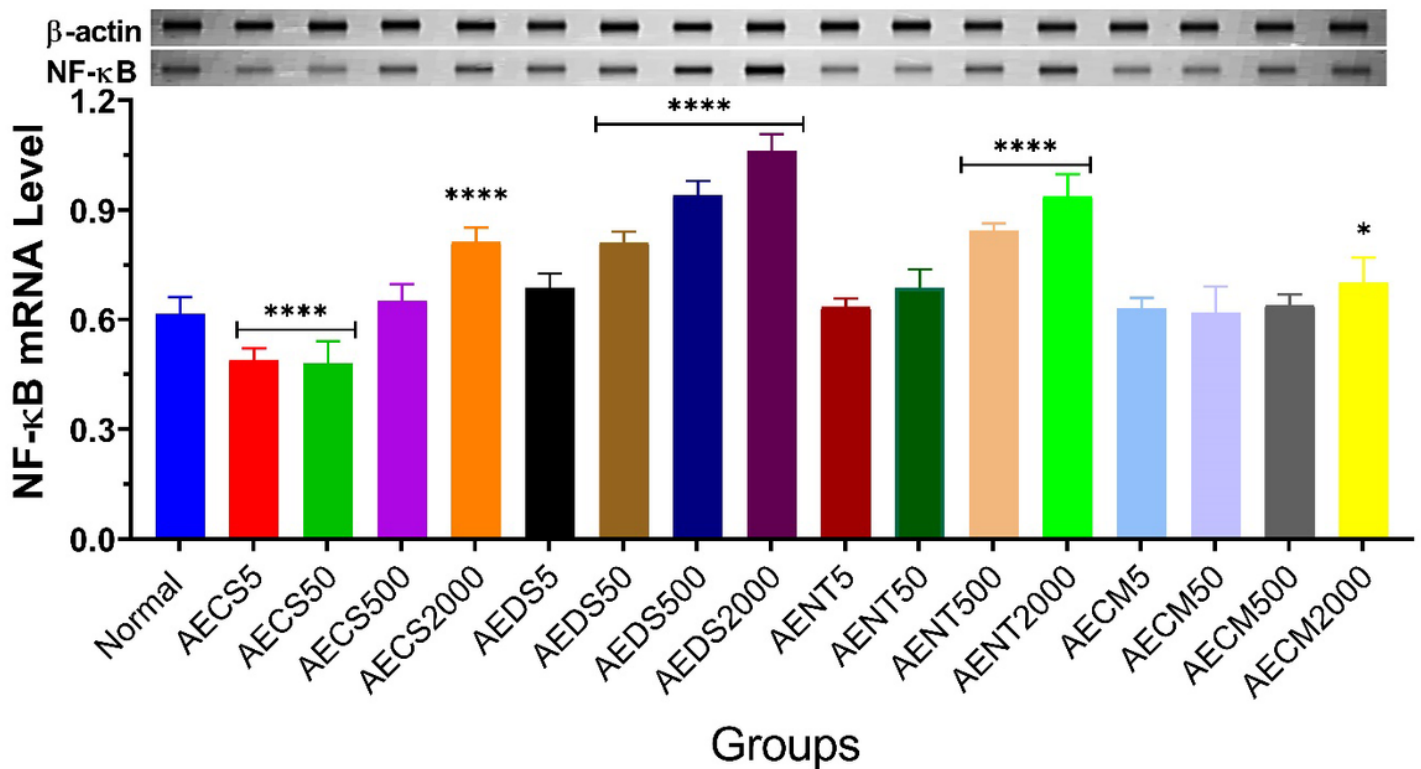


Figure 22

Effects of CSAE, DSAE, NTAE, and CMAE on Nuclear Factor-kappa B mRNA expression.

Results are expressed as mean $\pm$ SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group. AECS5 – orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; AECS50 – orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; AECS500 – orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; AECS2000 – orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; AEDS5 – orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; AEDS50 – orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; AEDS500 – orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; AEDS2000 – orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; AENT5 – orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; AENT50 – orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; AENT500 – orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; AENT2000 – orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; AECM5 – orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; AECM50 – orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; AECM500 – orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; AECM2000 – orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

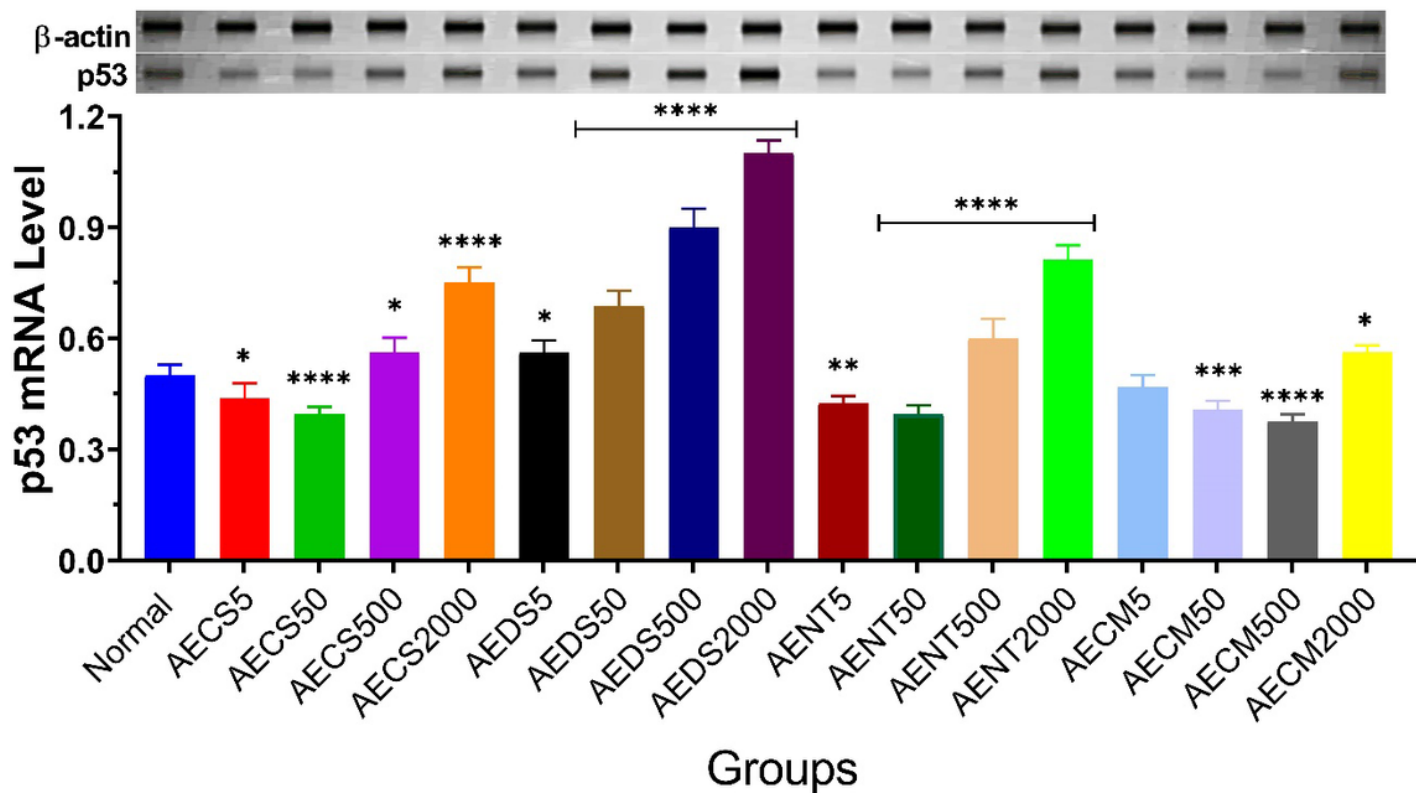


Figure 23

Effects of CSAE, DSAE, NTAE, and CMAE on p53 mRNA expression.

Results are expressed as mean $\pm$ SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with the normal control group. AECS5 – orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; AECS50 – orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; AECS500 – orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; AECS2000 – orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; AEDS5 – orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; AEDS50 – orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; AEDS500 – orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; AEDS2000 – orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; AENT5 – orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; AENT50 – orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; AENT500 – orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; AENT2000 – orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; AECSM5 – orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; AECSM50 – orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; AECSM500 – orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; AECSM2000 – orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.