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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 disorder that is characterized by habitual, compulsive, and pathological patterns of substance seeking and usage despite the associated severe negative social and health consequences.

Objective: This study evaluated the addictive and neurotoxicological evaluation of the alkaloid extracts of *Cannabis sativa* (CS), male *Carica papaya* (CM), *Nicotiana tabacum* (NT), and *Datura stramonium* (DS) via neuroinflammatory, neurotransmitter, and oxidative systems. For 90 days, experimental rats were orally administered the alkaloid extracts in doses of 5, 50, 500, and 2000 mg/kg. Neurobehavioral paradigms were evaluated on days 91 and 92, rats were sacrificed, and striatum homogenate was prepared. Expression of addition and neurotoxicity-related genes, alongside biochemical neurotransmitter and cytokine metabolisms, were assessed.

Results: The result established the addictive and neurotoxicological potentials of the alkaloid extracts via behavioral paradigms, coupled with inflammatory, monoaminergic, apoptotic, cholinergic, oxidative, and glutamatergic neurotransmission systems modulations. However, the observed neurotoxicity of the psychoactive plants' alkaloids was not directly proportional to their addictiveness as the psychoactive plants ranked CS > NT > DS > CM in addictiveness but ranked DS > NT > CS > CM for toxicological potentials, as measured using related behavioral, neurotransmitter, apoptotic and inflammatory systems.

Conclusion: The toxicological effects of the psychoactive plants' alkaloids are mostly expressed at high doses.

1. Introduction

Drug addiction is a relapsing and chronic brain disorder that is characterized by habitual, compulsive, and pathological patterns of substance seeking and usage despite the associated severe negative social and health consequences. An estimated 10 % of the global population is 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, 2019; UNODC, 2018, 2019; Citaristi, 2022). Drug addiction leads to neurological impairments and prioritizing the recreational use of psychoactive substances over life-sustaining activities and goals. It results in short- and long-term social, neurological, and health problems. Globally, the death of about 1.8 million individuals is reported every year (Cunha-Oliveira et al., 2008; Roth et al., 2018; Fasakin et al., 2022a). Regrettably, the highest proportion of the 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, 2019).

Plants with these addictive properties are known as psychoactive plants, with examples including *Cannabis sativa*, male *Carica papaya, Nicotiana tabacum,* and *Datura stramonium*. The alkaloids of these psychoactive plants have been implicated in their psychoactive, reinforcing, and rewarding effects (Jean-Francois, 2014; Alrashedy and Molina, 2016; Tan et al., 2021; Fasakin et al., 2022b; Youssef, 2024). In addition to pleasure incentives, the perception that the plants' constituents can enhance sexual performance, and alleviate anxiety, depressive moods, and hypertensive conditions (Fasakin et al., 2022a, 2022b, 2022c), has further escalated the plants' prevalent use, as the treated individuals continue the use of the plants after recovery from the treated-ailment

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(Müller et al., 2020). Addiction to these psychoactive plants has been observed to come in diverse biological phases (intoxication, withdrawal, bingeing, craving). 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.) that are key to existence (Cunha-Oliveira et al., 2013; Fasakin et al., 2022b).

The chronic use of these psychoactive plants has been suggested to result in addiction via stimulation of dopaminergic and glutamatergic pathways. However, overstimulation of these pathways has been reported to induce neurotoxicity directly via apoptosis, oxidative stress generation, neurocognitive impairments, and energy metabolism dysregulation, as well as trigger abnormal immune responses to deteriorate normal neuronal processes (Wang et al., 2019; Fasakin et al., 2022b; Niedzielska-Andres et al., 2022). Neuroimmune response to addiction is characterized by microglia stimulation that results in elevated proinflammatory cytokines, reactive nitrogen, and oxygen species production, which interacts with neurotransmitters in related brain circuits (Flores-López et al., 2021). Aggravated cytosolic dopamine couples with generated oxidative stress to evoke microglial cell activation, which causes neurotoxic damage via inflammatory cytokines (Nuclear Factor-kappa B, interleukin-1β and tumor necrosis factor-α) release and reactive oxygen species production (Turan et al., 2023). Remarkably, this biochemical pathway is a major neurobiological process explored by psychoactive substances during the development of addiction (Morcuende et al., 2021). Meanwhile, there is still a dearth of knowledge on the neurochemical link between the addictive and neurotoxicological profiles of most psychoactive plants' constituents. Interestingly, the study of da Silva et al. (2021) showed this neurochemical link as the pivot to improving risk assessment and drug use disorder treatments. Thus, this study seeks to evaluate the neurochemical link between the addictive and neurotoxicological profiles of *Cannabis sativa*, male *Carica papaya, Nicotiana tabacum,* and *Datura stramonium* via neuroinflammatory, neurotransmitter, and oxidative systems.

2. Materials and methods

2.1. Chemical and reagents

Acetylthiocholine iodide, adenosine, adenosine monophosphate, and Ellmans' reagent, were procured from Sigma-Aldrich (Germany). VWR Life Science (Solon, United States of America) and Zymo Research (United States of America) supplied TRI Reagent® and Nuclease-free water, respectively. BioLabs (New England) supplied the ProtoScript II First Strand cDNA Synthesis Kit and Luna Universal qPCR Master Mix, while Inqaba Biotechnology (Hatfield, South Africa) supplied the primers used in the present study.

2.2. Sample collection and extract preparation

Leaves of *Cannabis sativa*, male *Carica papaya* and *Nicotiana tabacum*, and *Datura stramonium* seeds were obtained from uncultivated farmlands in Akure South Local Government of Ondo State in Nigeria. They were identified and authenticated by the Centre for Research and Development (CERAD) unit of the Federal University of Technology, Akure (FUTA), Nigeria, with voucher numbers - 0346, 0347, 0348, and 0349. The leaves and seeds were carefully cleansed of stalks, debris, and other unwanted materials, sundry, and blended into powder using an electric blender. Alkaloid extracts were prepared using standard procedures (Fasakin et al., 2021). Typically, 100 g of powdered samples were defatted for 24 h with n-hexane. 200 mL of 10 % acetic acid in ethanol was added to the defatted samples and shaken vigorously; the mounting pressure was vented and allowed to stand for 24 h to permit sufficient extraction. The mixtures were filtered with a muslin cloth, filtered with Whatman No.1 filter paper, and concentrated using a rotary evaporator (Laborota 4000 Efficient, Heidolph, Germany) at 45 °C. A Dropwise concentration of ammonium hydroxide was added to the concentrated filtrate to achieve a good precipitate. The precipitate was harvested after allowing the whole solution to settle down to obtain the alkaloid extracts. Resultant alkaloid extracts were then termed CS (alkaloid extract of *Cannabis sativa*), NT (alkaloid extract of *Nicotiana tabacum*), DS (alkaloid extract of *Datura stramonium*), and CM (alkaloid extract of male *Carica papaya*), and were stored frozen for further analysis at 4 °C.

2.3. Experimental protocol

One hundred and two (102) male Wistar albino rats (weighing 178–187 g) were procured for the present study. The male Wistar rats were contained in plastic cages with controlled light of 12 h light and dark daily, relative humidity (60–70 %), and room temperatures (25–27 °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 (6) experimental animals per group were then randomly selected as described by Fasakin et al. (2022a), while extract dosages were selected according to the guidelines of Féres et al. (2006) and the Organization for Economic Co-operation and Development (2008):

Group 1 comprised normal experimental rats.
Groups 2–5 received 5, 50, 500, and 2000 mg/kg of CS.
Groups 6–9 received 5, 50, 500, and 2000 mg/kg of DS.
Groups 10–13 received 5, 50, 500, and 2000 mg/kg of NT.
Groups 14–17 received 5, 50, 500, and 2000 mg/kg of CM.

Alkaloid extracts were administered to experimental animals at selected dosages for ninety (90) days via oral gavage between 09:00 and 11:00 daily. Throughout the experimental period, experimental animals were allowed free access to water and rat chow ad libitum.

2.4. Behavioral tests

Experimental rats were exposed to conditioned place preference, and Y-Maze tests before the commencement of the experiment to establish the animals' cognitive status. After 90 days of oral exposure to alkaloid extracts at 5, 50, 500, and 2000 mg/kg, experimental rats were exposed to behavioral tests (conditioned place preference on day 91 and Y-Maze on day 92) to establish the addictive and neurotoxicological potentials of the alkaloid extracts during oral administration (Fig. 1). Experimental apparatuses were thoroughly cleaned with isopropyl alcohol after each animal session to remove olfactory cues.

2.4.1. Conditioned place preference test

Conditioned place preference (CPP) was conducted to assess the addictive potentials of the extracts using the modified methods of Do Couto et al. (2005) and Möller et al. (2018). The CPP apparatus comprised two compartments of 30 (height) x 20 (width) x 20 (length) with different visually (stripes/plain) colored walls and textured (rough/smooth) floor designs separated by a removable guillotine door. The CPP paradigm comprised three different stages of habituation (subjection of experimental animals to CPP apparatus before commencing the 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 an experimental rat in a vehicle-paired compartment and allowed free access to both compartment, a day after the last administration) stages for 20 minutes each per session, which was video-recorded under a dim white dispersed light and analyzed. Addictive behaviors were then calculated using the following formula:

Preference Index = Time spent in the drug-paired compartment during post-test – Time spent in drug paired-paired compartment during

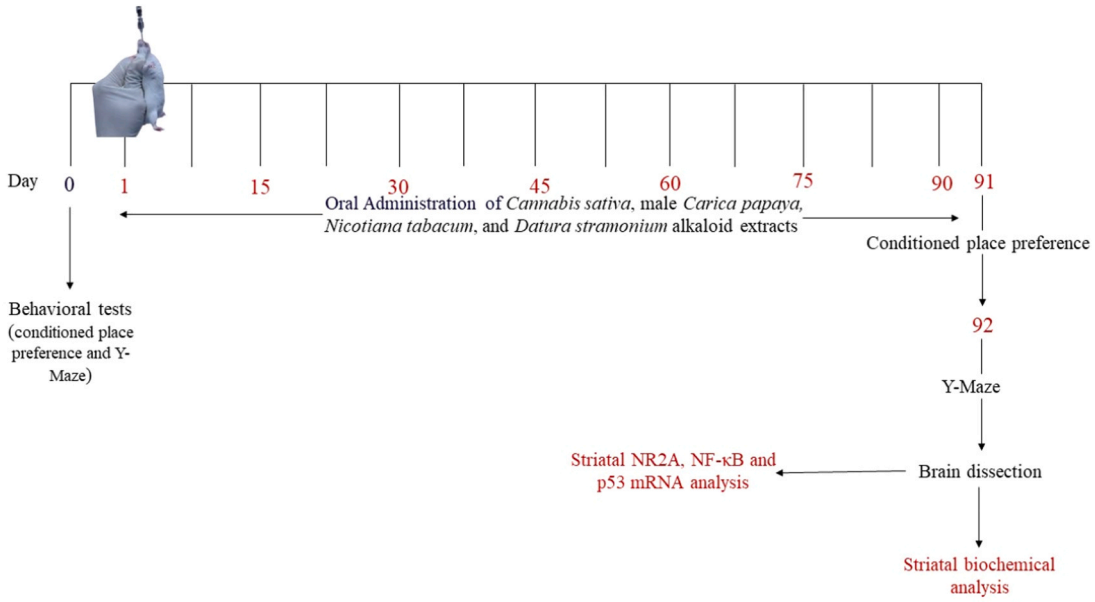

Fig. 1. Graph of Conducted Experimental Procedures.

habituation

Most or least time spent in the drug-paired compartment was then used in determining the addictive or aversive potentials of the alkaloid extracts respectively.

2.4.2. 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). Experimental rats were positioned with their backs to the edge of a Y-maze apparatus and given five minutes to decide movements between the Y-maze arms. The memory index (% of correct alteration) was calculated by recording the frequencies of correct movements (ABC, ACB, BCA, BAC, CBA, or CAB) between the arms (ABC). The memory index is presented as a function of the percentage alternation between successive arms of the maze.

$$\text{Memory Index} = \frac{\text{Number of right decisions}}{\text{Total arm entries} - 2} \quad \text{x } 100$$

2.5. Tissue homogenate preparation

After the behavioral study, the experimental animals were anesthetized using mild diethyl ether, sacrificed and the brain removed while the striatum was carefully removed for subsequent analysis (Fasakin et al., 2022a).

2.6. Gene expression analysis

Total ribonucleic acid was extracted from the striatal tissues using TRI Reagent® while the relative cDNA amount of N-methyl-D-aspartate receptor subunit 2 A (NR2A), Nuclear Factor-kappa B, and p53 was quantified as previously reported by Fasakin et al. (2022a) with the 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	AGCCCCCTTCGTCATCGTAGA-Forward
subunit 2A (NR2A)	ACCCCTTGCAGCACTTCTTCAC-Reverse
Nuclear Factor-kappa B	AGACATCCTTCCGCAAACTC-Forward
	TAGGTCCATCCTGCCCATAA-Reverse
p53	ACGTGATGAGGATGGGAAAC-Forward
	TATCTGGCTTCTTGCTCTTGG-Reverse

2.7. 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. Striatal dopamine concentrations were evaluated at 735 nm using 5 mM $FeCl_3$ and dopamine hydrochloride (Guo et al., 2009). Acetylcholinesterase (AChE) activity was evaluated using AChE as substrate (Perry et al., 2000). Abdel-Zaher et al. (2011) method was employed to evaluate glutamate dehydrogenase activity. Hayashi et al. (2007) method was employed to quantify reactive oxygen species (ROS) production. Nitric oxide concentration was evaluated using Green et al. (1982). The activity of lactate dehydrogenase (LDH) and concentrations of interleukin-1β (IL-1β) and interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α) were evaluated using commercially available Enzyme-Linked Immunosorbent Assay kits.

2.8. Statistical analysis

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

3. Results

3.1. Effects of CS, DS, NT, and CM on experimental rats behavioral paradigms

The effects of the alkaloid extracts were evaluated for addiction and neurotoxicity using conditioned place preference (CPP), and Y-maze tests.

The result showed that animals administered 5, 50, and 500 mg/kg body weight of CS, NT, and CM expressed significant ($P < 0.0001$; $F_{(16,85)} = 631.3$) alternation in preference index during CPP tests (Fig. 2). The administration of CS, DS, NT, and CM elicited a dose-

Table 2
GC–MS characterization of CS.

S/N	RT(min)	Compound Name	Mol. Formula	MW	Peak Area %	Comp (μg/g)
1	10.82	Choline	$C_5H_{14}NO^+$	104	2.23	23.03
2	13.41	Trigonelline	$C_7H_7NO_2$	137	3.59	20.41
3	14.00	Dopamine	$C_8H_{11}NO_2$	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	$C_{20}H_{25}N_3O$	323	3.03	50.04
10	35.60	Boldine	$C_{19}H_{21}NO_4$	327	5.75	103.41
11	41.50	Strychnine	$C_{21}H_{22}N_2O_2$	334	8.62	72.63
12	43.00	Anhydrocannabisativine	$C_{21}H_{37}N_3O_2$	363	12.25	218.11
13	44.31	Cannabisativine	$C_{21}H_{39}N_3O_3$	381	17.24	410.30
14	46.78	Cannabimine C	$C_{21}H_{37}N_3O_2$	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 DS.

S/N	RT (min)	Compound Name	Mol. Formula	MW	Peak Area %	Comp (μg/g)
1	7.50	2-Pyrrolidinone	C_4H_7NO	85	6.15	110.24
2	7.81	8-Methyl−8-azabicyclo[3.2.1]octane−2,6-diol	$C_8H_{15}NO_2$	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)	$C_{20}H_{26}N_2O_2$	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-dioxopyridazino[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

Table 4
GC–MS characterization of NT.

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)-	$C_9H_{10}N_2$	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-	$C_{10}H_9N$	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	$C_9H_8N_2$	144	3.66	11.36
7	18.57	2,2′-Bipyridine	$C_{10}H_8N_2$	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	$C_{10}H_8N_2$	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	$C_{10}H_{12}N_2O$	176	3.05	14.90
12	34.21	N-Nitrosonornicotine	$C_9H_{11}N_3O$	177	5.00	12.00
13	38.76	Nornicotine, N-formyl	$C_{10}H_{12}N_2O$	176	6.08	18.04
14	42.50	2-Pyrrolidinone, 3-hydroxy−1-methyl−5-(3-pyridinyl)-, (3R-trans)	$C_{10}H_{12}N_2O_2$	192	7.39	10.00
15	46.50	Nornicotine, N-acetyl	$C_{11}H_{14}N_2O$	190	10.99	11.15

dependent response as shown in Fig. 5 after the administration of the extracts for 90 days. The obtained result showed significantly ($P < 0.0001$; $F_{(16,85)} = 305.9$) improved preference index for CS, NT, and CM, whereas negative result was obtained for DS at 500 and 2000 mg/kg body weight, which may be suggestive of aversiveness at the dosages.

Also, the rats elicited a dose-dependent response ($P < 0.0001$; $F_{(16,85)} = 128.7$) spatial working memory (Y-maze test) after the sub-chronic administration of CS, NT, and CM in comparison to the control group (Fig. 3). The administration of DS depleted spontaneous alternations in comparison to the control group, an indication of reduced spatial memory function.

Table 5
GC–MS characterization of CM.

S/N	RT (min)	Compound Name	Mol. Formula	MW	Peak Area %	Comp (μg/g)
1	3.81	Choline	$C_5H_{14}NO^+$	104	0.88	7.04
2	5.25	8-Methyl−8-azabicyclo[3.2.1] octane−2,6-diol	$C_8H_{15}NO_2$	157	0.97	8.05
3	11.18	Benzylthiourea	$C_8H_{10}N_2S$	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	$C_7H_7NO_2$	137	14.07	57.06
7	14.20	+)-Laurotetanine	$C_{19}H_{21}NO_4$	327	12.40	120.54
8	15.76	Pseudocarpaine	$C_{28}H_{50}N_2O_4$	478	10.55	412.92
9	16.25	Carpaine	$C_{28}H_{50}N_2O_4$	478	19.27	514.36
10	16.50	Alstoniaphylline C	$C_{20}H_{24}N_2O_3$	350	10.34	40.38
11	17.32	(+)-alstonisine	$C_{20}H_{22}N_2O_3$	338	6.16	152.90
12	18.00	Dehydrocarpaine I	$C_{28}H_{48}N_2O_4$	476	4.40	445.18
13	20.42	Dehydrocarpaine II	$C_{28}H_{46}N_2O_4$	434	5.32	274.71

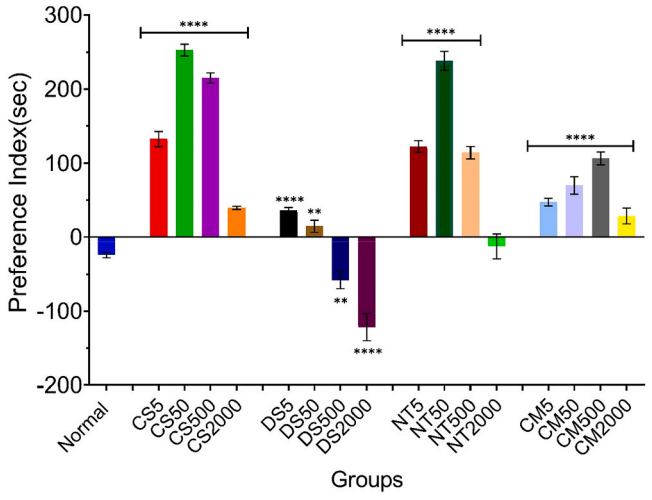

Fig. 2. Effects of CS, DS, NT, and CM on preference index during conditioned place preference (CPP). Results are expressed as mean ± SEM (n = 6). *p < 0.05, * *p < 0.01, * **p < 0.001, * ** *p < 0.0001 compared with the normal control group. CS5 — orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; CS50 — orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; CS500 — orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; CS2000 — orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; DS5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; DS50 — orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; DS500 — orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; DS2000 — orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; NT5 — orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; NT50 — orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; NT500 — orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; NT2000 — orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; CM5 — orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; CM50 — orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; CM500 — orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; CM2000 — orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

3.2. Effects of CS, DS, NT, and CM on the striatal neurotransmission system

The effect of sub-chronic exposure of the alkaloid extracts on neurotransmitter metabolism is shown in Figs. 4 – 9. 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 exposure to CS, DS, NT, and CM (Figs. 4 – 9). Striatal dopaminergic and nitric oxide concentrations were significantly ($P < 0.0001$; $F_{(16,85)} = 81.63$) and ($P < 0.0001$; $F_{(16,85)} = 98.86$) increased after the administration of CS, NT, and CM in a dose-dependent response (Figs. 10 and 11). Interestingly, the concentration of NO was consistent with that observed for dopamine concentration, which is suggestive of the link between the two neurotransmission systems.

3.3. Effects of CS, DS, NT, and CM on the striatal inflammatory system

The effect of sub-chronic exposure of the alkaloid extracts on inflammatory concentrations is shown in Figs. 12 – 14. Results showed that the concentrations of α-tumor necrosis factor ($P < 0.0001$; $F_{(16,85)} = 30.94$) and IL-1β ($P < 0.0001$; $F_{(16,85)} = 119.8$) were depleted while IL-10 concentrations ($P < 0.0001$; $F_{(16,85)} = 94.62$) was elevated during sub-chronic exposure to CS, NT, and CM. However, DS administration elevated TNFα and IL-1β concentrations while decreasing IL-10 concentration.

3.4. Effects of CS, DS, NT, and CM on striatal redox stress and lactate dehydrogenase activity

The effect of sub-chronic exposure of the alkaloid extracts on redox stress is shown in Fig. 15. Results observed that sub-chronic exposure to CS, NT, and CM inhibited reactive oxygen species production at lower doses ($P < 0.0001$; $F_{(16,85)} = 260.0$), while at 2000 mg/kg, elevated ROS production was observed (Fig. 15). All doses of DS increased reactive oxygen species production. The CS and NT stimulated a dose-dependent 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. 16). The administration of CM and DS caused a significant ($P < 0.05$) reduction and elevation, respectively in the activities of the enzyme when compared to control rats.

3.5. Effects of CS, DS, NT, and CM on gene expression

Figs. 17 – 19 showed the modulatory effect of CS, DS, NT, and CM on striatal gene expressions. The result showed that CS, NT, and CM administration elicited a significant ($P < 0.05$) elevation in the striatal mRNA expressions of N-methyl-D-aspartate receptor subunit 2 A ($P < 0.0001$; $F_{(16, 85)} = 188.0$) (Fig. 17). Also, the result showed that CS and CM administration caused a concentration-dependent modulation in striatal and hippocampal mRNA expressions of Nuclear Factor-kappa B ($P < 0.0001$; $F_{(16, 85)} = 72.95$) and p53 ($P < 0.0001$; $F_{(16, 85)} = 210.5$) (Figs. 18 and 19).

4. Discussion

In understanding the neurobiology of addiction and the neuropharmacological action of psychoactive plants, the conditioned place preference (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). Thus, the results of this study indicate that CS, DS, NT, and CM may have addictive potential via reinforcing and rewarding effects (Hur et al., 2020). The improved ability of alkaloid extracts-administered animals to make correct alterations, and maintain movement orderliness during the Y-maze, except for DS (50, 500, and 2000) and NT2000

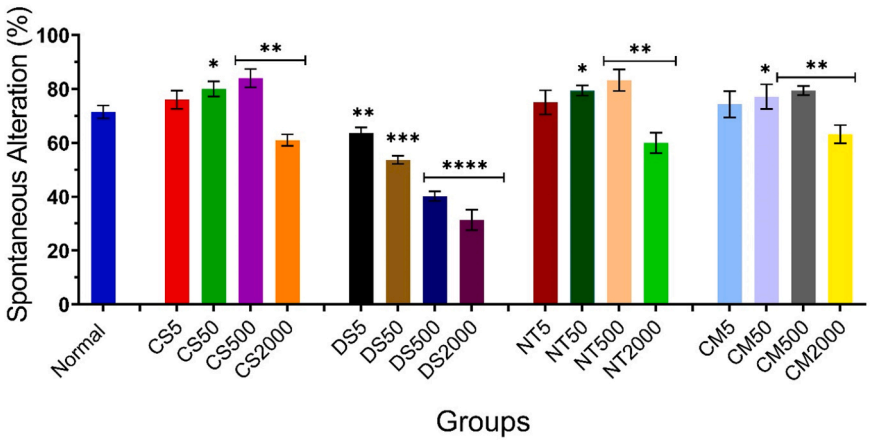

Fig. 3. Effects of CS, DS, NT, and CM on spontaneous alternation using Y-Maze. Results are expressed as mean ± SEM (n = 6). *p < 0.05, * *p < 0.01, * **p < 0.001, * ** *p < 0.0001 compared with the normal control group. CS5 — orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; CS50 — orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; CS500 — orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; CS2000 — orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; DS5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; DS50 — orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; DS500 — orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; DS2000 — orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; NT5 — orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; NT50 — orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; NT500 — orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; NT2000 — orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; CM5 — orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; CM50 — orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; CM500 — orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; CM2000 — orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

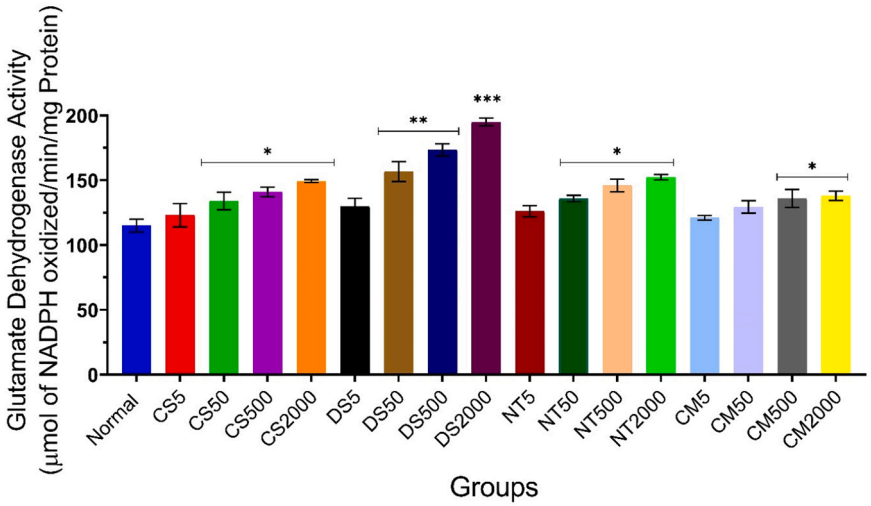

Fig. 4. Effects of CS, DS, NT, and CM on Glutamate dehydrogenase Activities. Results are expressed as mean ± SEM (n = 6). *p < 0.05, * *p < 0.01, * **p < 0.001, * ** *p < 0.0001 compared with the normal control group. CS5 — orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; CS50 — orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; CS500 — orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; CS2000 — orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; DS5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; DS50 — orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; DS500 — orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; DS2000 — orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; NT5 — orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; NT50 — orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; NT500 — orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; NT2000 — orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; CM5 — orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; CM50 — orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; CM500 — orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; CM2000 — orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

may indicate enhanced cognitive functions, spatial memory, and learning improvements, which can be linked to the observed CCP paradigm patterned observed in the present study (Šlamberová et al., 2015). Interestingly, this result trend was observed with morphine by Markos et al. (2018). Inferentially, the administration of DS (50, 500, and 2000) and NT2000 may pose neurotoxic effects on the striatum of experimental rats despite the memory improvement observed with lower doses of the same alkaloids.

The GC–MS characterization of the alkaloid extracts as reported in our previous study of Fasakin et al. (2022a) showed that CS contains choline, Cannabidiol, Δ-9-tetrahydrocannabinol, Cannabisativine, Cannabimine C, Aconitine and others. However, it is Δ-9-tetrahydrocannabinol (ΔTHC), which interacts with the CB1 receptor in the human brain, that has been implicated in *C. sativa's* addictive potential. The interaction between ΔTHC and CB1 receptors generates signals that activate G-proteins, which further transfer the signal to activate or inhibit various transduction pathway-related signals. Meanwhile, G-protein plays a direct role in blocking voltage-gated calcium and

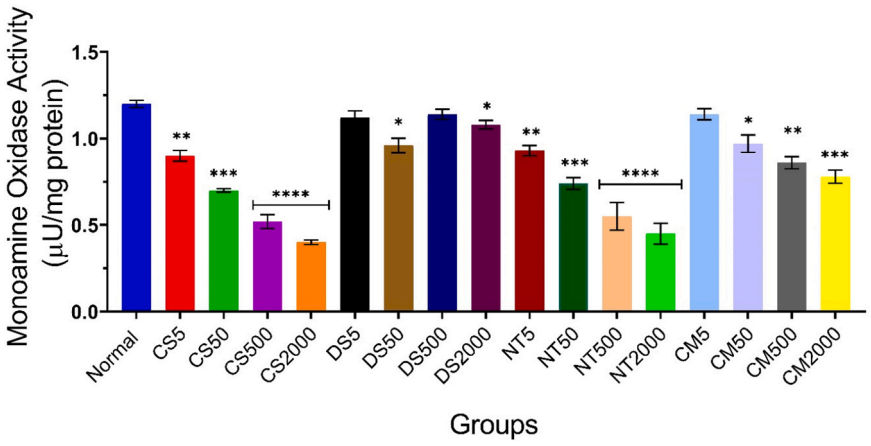

Fig. 5. Effects of CS, DS, NT, and CM on Monoamine Oxidase Activities. Results are expressed as mean ± SEM (n = 6). *p < 0.05, * *p < 0.01, * **p < 0.001, * ** *p < 0.0001 compared with the normal control group. CS5 — orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; CS50 — orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; CS500 — orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; CS2000 — orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; DS5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; DS50 — orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; DS500 — orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; DS2000 — orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; NT5 — orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; NT50 — orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; NT500 — orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; NT2000 — orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; CM5 — orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; CM50 — orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; CM500 — orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; CM2000 — orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

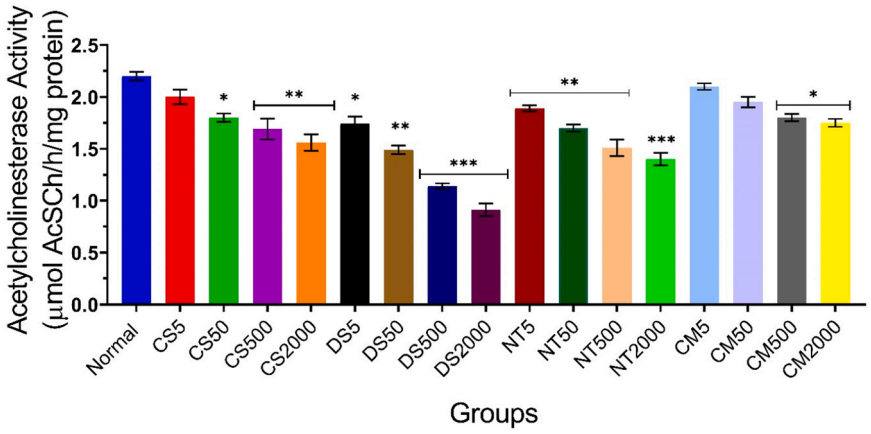

Fig. 6. Effects of CS, DS, NT, and CM on Acetylcholinesterase Activities. Results are expressed as mean ± SEM (n = 6). *p < 0.05, * *p < 0.01, * **p < 0.001, * ** *p < 0.0001 compared with the normal control group. CS5 — orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; CS50 — orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; CS500 — orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; CS2000 — orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; DS5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; DS50 — orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; DS500 — orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; DS2000 — orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; NT5 — orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; NT50 — orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; NT500 — orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; NT2000 — orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; CM5 — orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; CM50 — orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; CM500 — orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; CM2000 — orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

sodium channels, which when blocked, causes the mitogen-activated protein kinase pathway to be activated, and resultantly neurotoxicity (Datta et al., 2021). Atropine, Hyoscyamine, Scopolamine, Anisodine, 2-pyrrolidinone, and others were observed in DS, however, it is the tropane alkaloids (scopolamine and atropine) that are central nervous system modulators, producing hallucination and depression (Ademiluyi et al., 2016). Fasakin et al. (2022b) noted that atropine stimulates the central nervous system, while scopolamine calms the central nervous system. They have also been implicated in the neurotoxic effects of the plant, *D. stramonium*. Pyridine, nornicotine, anatabine, anabasine, nicotine, cotinine, quinoline, nornicotyrine, nicotyrine, and others were observed in NT, however, it is the potent parasympathomimetic alkaloids (nicotine, cotinine, anabasine, and nornicotine) that synergistically work to stimulate addiction. The study of Kuete (2014) showed that these parasympathomimetic alkaloids (especially nicotine and anabasine) act as a stimulant at low doses but as a neurotoxicant at high doses. Anonaine, trigonelline, pseudocarpaine, carpain, choline, alstoniaphylline C, dehydrocarpaine I, dehydrocarpaine II, and others were observed in CM, however, it is only carpain and choline that have been reported for their addictive effects. Carpain forms hydrogen bonds and hydrophilic interactions with the choline (acetyl- and butyryl-) acyl pocket domains and binding sites, resulting in significant inhibition of

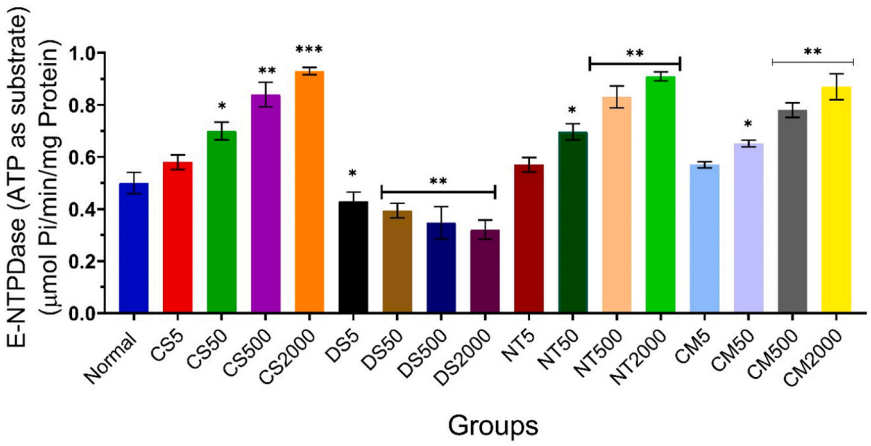

Fig. 7. Effects of CS, DS, NT, and CM on E-NTPDase (ATP) Activities. Results are expressed as mean ± SEM (n = 6). *p < 0.05, * *p < 0.01, * **p < 0.001, * ** *p < 0.0001 compared with the normal control group. CS5 — orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; CS50 — orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; CS500 — orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; CS2000 — orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; DS5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; DS50 — orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; DS500 — orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; DS2000 — orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; NT5 — orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; NT50 — orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; NT500 — orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; NT2000 — orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; CM5 — orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; CM50 — orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; CM500 — orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; CM2000 — orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

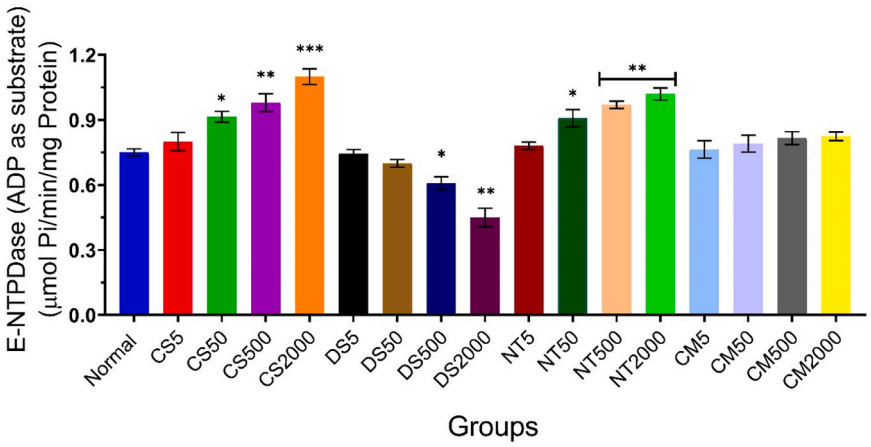

Fig. 8. Effects of CS, DS, NT, and CM on E-NTPDase (ADP) Activities. Results are expressed as mean ± SEM (n = 6). *p < 0.05, * *p < 0.01, * **p < 0.001, * ** *p < 0.0001 compared with the normal control group. CS5 — orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; CS50 — orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; CS500 — orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; CS2000 — orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; DS5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; DS50 — orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; DS500 — orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; DS2000 — orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; NT5 — orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; NT50 — orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; NT500 — orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; NT2000 — orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; CM5 — orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; CM50 — orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; CM500 — orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; CM2000 — orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

cholinesterase activities. This results in excessive choline accumulation at the synaptic cleft at higher exposure doses and ultimately, neurodegeneration (Khaw et al., 2020; Fasakin et al., 2022b). Thus, in comparison to previously established findings, the present study points to the ability of the CS, DS, NT, and CM to trigger addiction, however, neurotoxicity was observed at high doses.

The depletion observed with the DS500, DS2000, and NT2000 groups in comparison with the normal control group during the CPP test may be dependent on the adverse effect observed with the Y-maze test, a validation that the achievement of conditioned place preference necessitates the application of memory and learning as previously observed by Itzhak and Ali (2006). This may also indicate the administrations of *D. stramonium* and *N. tabacum* alkaloids at those doses had lower drug cue preferences. 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 (González et al., 2005; Zernig et al., 2007; Hur et al., 2020; Fasakin et al., 2022b). Thus, the CS, DS, NT, and CM possess the potential to be both addictive and neurotoxic.

The striatal dopaminergic brain pathway is a key pathway in the psychoactive, addictive, and reinforcing effects of all psychoactive

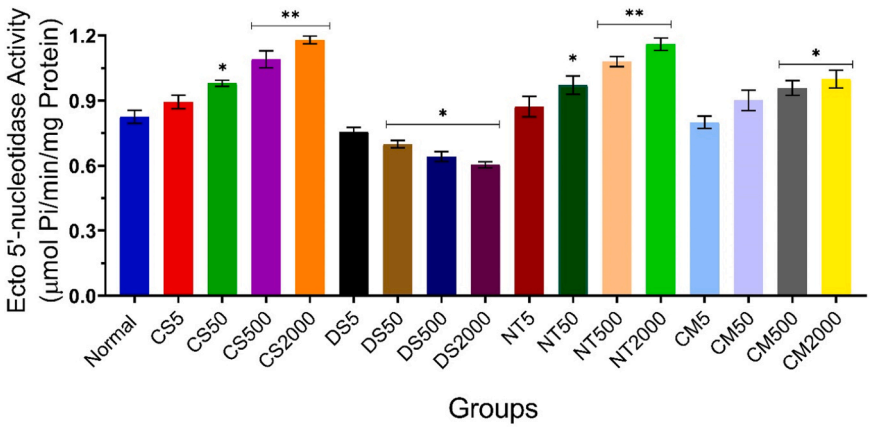

Fig. 9. Effects of CS, DS, NT, and CM on ecto-5'-nucleotidase Activities. Results are expressed as mean ± SEM (n = 6). *p < 0.05, * *p < 0.01, * **p < 0.001, * ** *p < 0.0001 compared with the normal control group. CS5 — orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; CS50 — orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; CS500 — orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; CS2000 — orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; DS5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; DS50 — orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; DS500 — orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; DS2000 — orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; NT5 — orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; NT50 — orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; NT500 — orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; NT2000 — orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; CM5 — orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; CM50 — orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; CM500 — orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; CM2000 — orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

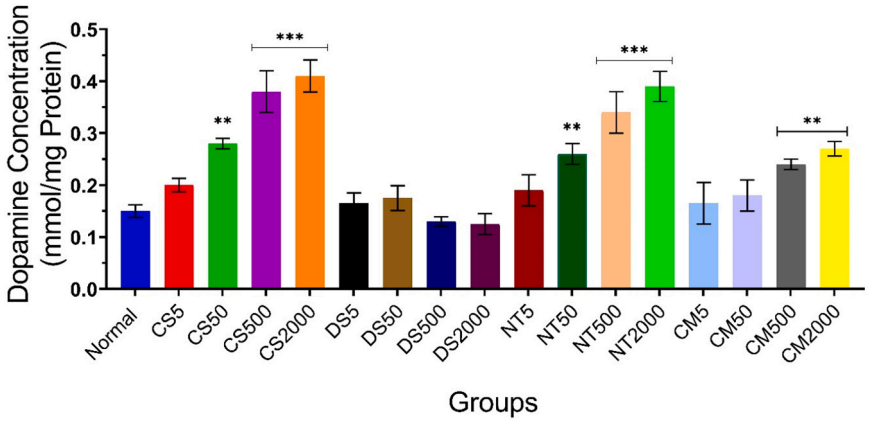

Fig. 10. Effects of CS, DS, NT, and CM on Dopamine Concentration. Results are expressed as mean ± SEM (n = 6). *p < 0.05, * *p < 0.01, * **p < 0.001, * ** *p < 0.0001 compared with the normal control group. CS5 — orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; CS50 — orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; CS500 — orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; CS2000 — orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; DS5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; DS50 — orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; DS500 — orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; DS2000 — orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; NT5 — orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; NT50 — orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; NT500 — orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; NT2000 — orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; CM5 — orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; CM50 — orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; CM500 — orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; CM2000 — orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

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 the activity of MAO, dopamine deaminating enzyme (Cunha-Oliveira et al., 2008; Fasakin et al. 2021). Therefore, the heightened inhibition of the MAO enzyme activity by CS, NT, and CM administration may have resulted in elevated striatal dopaminergic concentrations in the present study, which indicates significant memory improvement. However, the elevated LDH activities observed in the present study may indicate interference with the dopaminergic pathway to induce neurotoxicity as elevated LDH release in heightened dopamine-concentration neurons has been implicated by Asanuma et al. (2020) to be suggestive of the loss of cell membrane integrity. This suggests the ability of the CS, DS, NT, and CM to induce addition and neurotoxicity via stimulation of the dopaminergic neurotransmission system.

Glutamate, the major excitatory brain neurotransmitter, and its direct involvement in the addictive, drug-seeking, and dependence behaviors of drugs of abuse via the reward circuits in brain cells are 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,

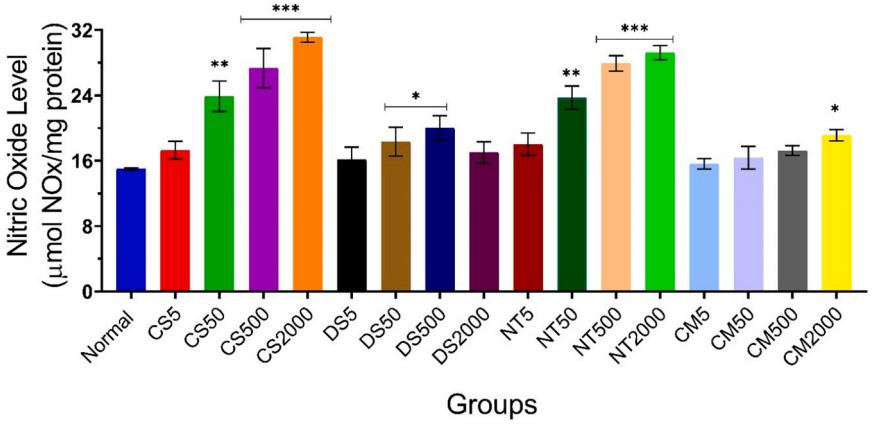

Fig. 11. Effects of CS, DS, NT, and CM on Nitric oxide Concentration. Results are expressed as mean ± SEM (n = 6). *p < 0.05, * *p < 0.01, * **p < 0.001, * ** *p < 0.0001 compared with the normal control group. CS5 — orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; CS50 — orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; CS500 — orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; CS2000 — orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; DS5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; DS50 — orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; DS500 — orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; DS2000 — orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; NT5 — orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; NT50 — orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; NT500 — orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; NT2000 — orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; CM5 — orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; CM50 — orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; CM500 — orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; CM2000 — orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

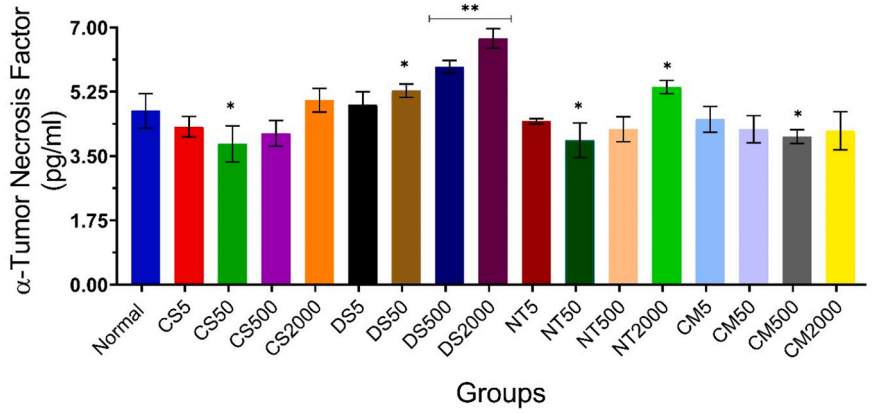

Fig. 12. Effects of CS, DS, NT, and CM on α-Tumour Necrosis Factor Concentration. Results are expressed as mean ± SEM (n = 6). *p < 0.05, * *p < 0.01, * **p < 0.001, * ** *p < 0.0001 compared with the normal control group. CS5 — orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; CS50 — orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; CS500 — orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; CS2000 — orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; DS5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; DS50 — orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; DS500 — orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; DS2000 — orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; NT5 — orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; NT50 — orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; NT500 — orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; NT2000 — orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; CM5 — orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; CM50 — orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; CM500 — orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; CM2000 — orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

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 NR1 subunit that consists of a glycine binding site and the other consisting of a glutamate binding site (Ehsanifar et al., 2019). The increase in glutamate dehydrogenase activity and the activation of NR2A during the present study may be indicative that the stimulation of glutamatergic NMDA receptors is involved in facilitating the addictive effects of the CS, DS, NT, and CM as previously observed by Josiah et al. (2021) and Fasakin et al. (2022a). However, overstimulation of the glutamatergic NMDA receptors has been implicated to result in mitochondria impairments and excitotoxicity (Myslivecek, 2021), which is suggestive of the neurotoxicological potential of DS at high doses. Thus, this study establishes the potency of CS, DS, NT, and CM to trigger addiction and neurotoxicity via the glutamatergic neurotransmission system.

The cholinergic neurotransmission system has been implicated in inducing 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 has also been established during the dependence and reinforcing-producing actions of nicotine by Lester et al. (2010) and Myslivecek (2021). The inhibitory

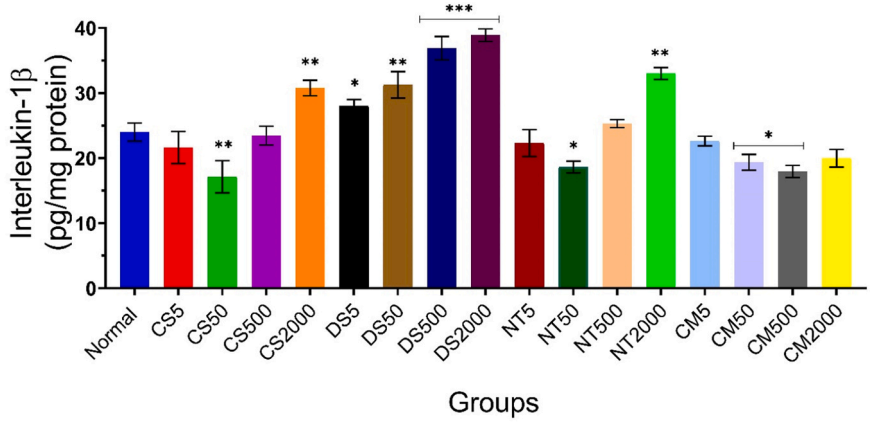

Fig. 13. Effects of CS, DS, NT, and CM on interleukin-1β Concentration. Results are expressed as mean ± SEM (n = 6). *p < 0.05, * *p < 0.01, * **p < 0.001, * ** *p < 0.0001 compared with the normal control group. CS5 — orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; CS50 — orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; CS500 — orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; CS2000 — orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; DS5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; DS50 — orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; DS500 — orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; DS2000 — orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; NT5 — orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; NT50 — orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; NT500 — orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; NT2000 — orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; CM5 — orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; CM50 — orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; CM500 — orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; CM2000 — orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

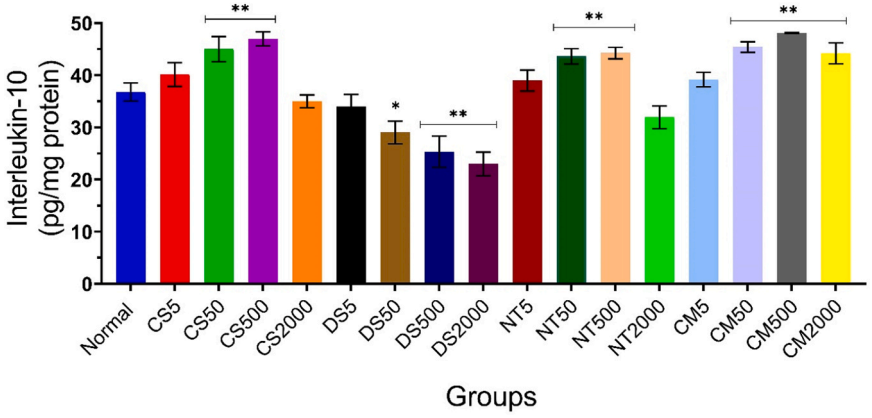

Fig. 14. Effects of CS, DS, NT, and CM on interleukin-10 Concentration. Results are expressed as mean ± SEM (n = 6). *p < 0.05, * *p < 0.01, * **p < 0.001, * ** *p < 0.0001 compared with the normal control group. CS5 — orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; CS50 — orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; CS500 — orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; CS2000 — orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; DS5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; DS50 — orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; DS500 — orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; DS2000 — orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; NT5 — orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; NT50 — orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; NT500 — orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; NT2000 — orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; CM5 — orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; CM50 — orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; CM500 — orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; CM2000 — orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

effects on acetylcholinesterase may be suggestive of the possible addictive mechanism of the CS, DS, NT, and CM, as acetylcholinesterase is the principal factor underlining upregulated cholinergic neurotransmission and homeostatic acetylcholine concentrations (Fasakin et al., 2022a; Ademosun et al., 2023). Extensive inhibition of acetylcholinesterase results in excess synaptic acetylcholine concentrations, altered postsynaptic cell function, overstimulated cholinergic receptors, and cholinergic toxicity (Pope et al., 2005). DS 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). Thus, the addictive and neurotoxic potentials of the CS, DS, NT, and CM can be established via the cholinergic neurotransmission system.

The high oxidizability of dopamine alongside high concentrations of dopamine observed in this study may have resulted in the aggravated ROS concentrations after CS, DS, NT, and CM administrations. The inappropriate sequestering of dopamine into vesicles results in high cytosolic dopamine concentrations that elevate 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 a 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

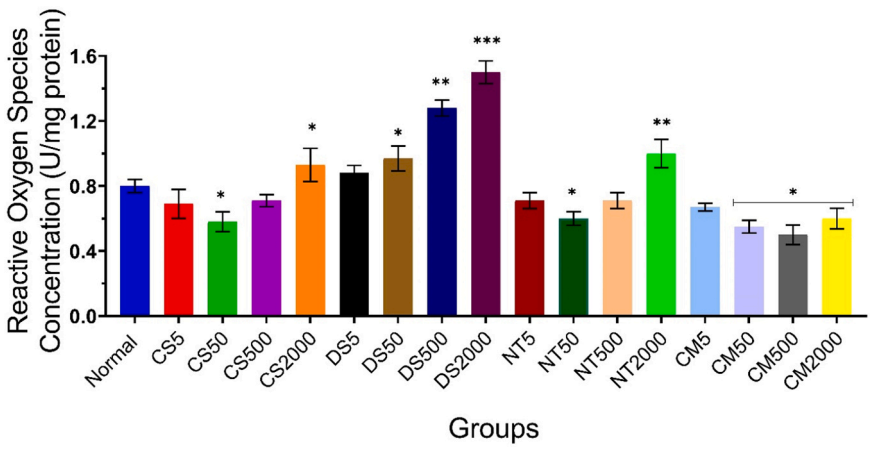

Fig. 15. Effects of CS, DS, NT, and CM on Reactive oxygen species production. Results are expressed as mean ± SEM (n = 6). *p < 0.05, * *p < 0.01, * **p < 0.001, * ** *p < 0.0001 compared with the normal control group. CS5 — orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; CS50 — orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; CS500 — orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; CS2000 — orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; DS5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; DS50 — orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; DS500 — orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; DS2000 — orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; NT5 — orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; NT50 — orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; NT500 — orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; NT2000 — orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; CM5 — orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; CM50 — orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; CM500 — orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; CM2000 — orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

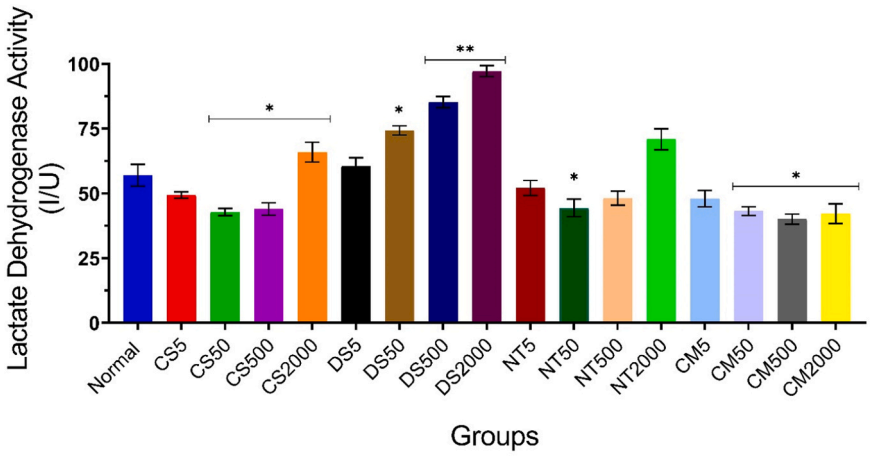

Fig. 16. Effects of CS, DS, NT, and CM on Lactate dehydrogenase Activities. Results are expressed as mean ± SEM (n = 6). *p < 0.05, * *p < 0.01, * **p < 0.001, * ** *p < 0.0001 compared with the normal control group. CS5 — orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; CS50 — orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; CS500 — orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; CS2000 — orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; DS5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; DS50 — orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; DS500 — orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; DS2000 — orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; NT5 — orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; NT50 — orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; NT500 — orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; NT2000 — orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; CM5 — orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; CM50 — orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; CM500 — orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; CM2000 — orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

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 ($O_2.^-$) production, with the latter having the ability to react with elevated nitric oxide concentrations to form highly neurotoxic peroxynitrite. Considerably, the ability of CS, DS, NT, and CM to significantly stimulate dopamine release and oxidative stress production may have better established the link between the addictiveness and neurotoxicological potential of the samples as previously reported by Cunha-Oliveira et al. (2008) for opioid and psychostimulant drugs.

The induced-oxidative stress can trigger inflammatory responses characterized by heightened upregulation of inflammatory mediators (e.g., TNF-α, NF-κB, IL-1β) and inhibition of anti-inflammatory mediators (e.g., IL-10) to stimulate apoptotic pathway (Fasakin et al., 2022a; Josiah et al., 2022). Neuroimmune response to addiction is characterized by the stimulation of microglia, which leads to elevated proinflammatory cytokine production that interacts with neurotransmitters in related brain circuits (Flores-López et al. 2021). Brain pro-inflammatory neuroimmune signaling has been established to regulate GABA and AMPA receptor trafficking during addiction (Gipson et al., 2021). The

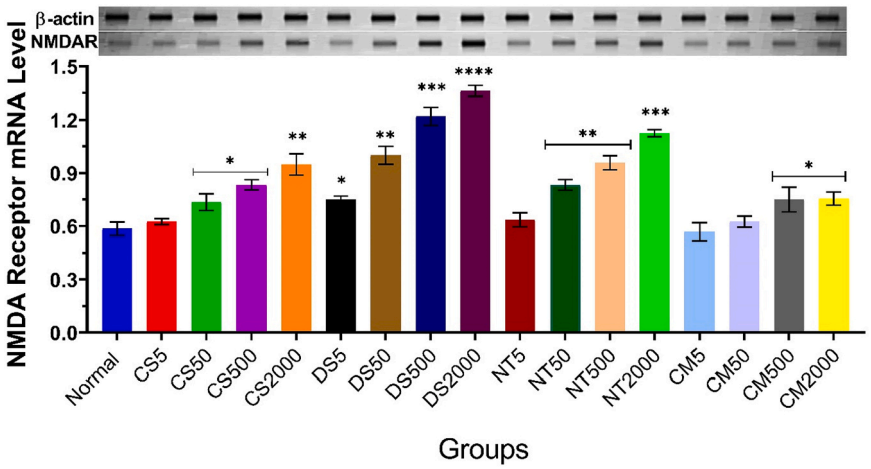

Fig. 17. Effects of CS, DS, NT, and CM on N-methyl-D-aspartate receptor subunit 2 A mRNA expression. Results are expressed as mean ± SEM (n = 6). *p < 0.05, * *p < 0.01, * **p < 0.001, * ** *p < 0.0001 compared with the normal control group. CS5 — orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; CS50 — orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; CS500 — orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; CS2000 — orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; DS5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; DS50 — orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; DS500 — orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; DS2000 — orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; NT5 — orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; NT50 — orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; NT500 — orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; NT2000 — orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; CM5 — orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; CM50 — orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; CM500 — orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; CM2000 — orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

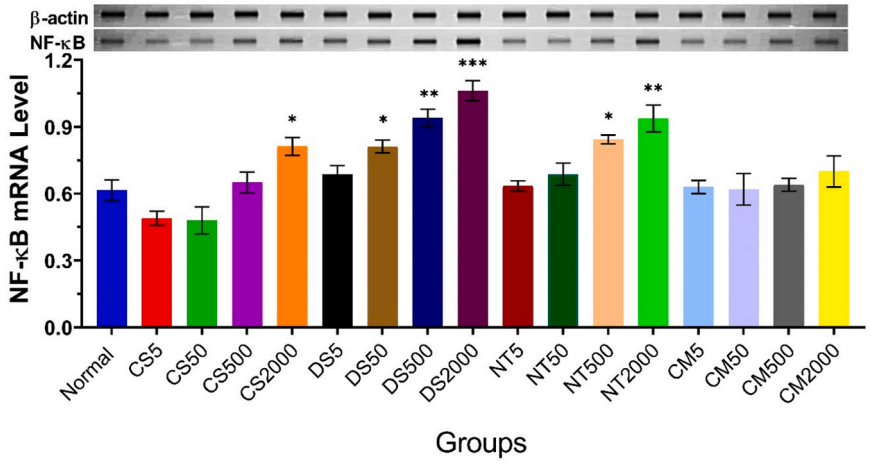

Fig. 18. Effects of CS, DS, NT, and CM on Nuclear Factor-kappa B mRNA expression. Results are expressed as mean ± SEM (n = 6). *p < 0.05, * *p < 0.01, * **p < 0.001, * ** *p < 0.0001 compared with the normal control group. CS5 — orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; CS50 — orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; CS500 — orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; CS2000 — orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; DS5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; DS50 — orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; DS500 — orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; DS2000 — orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; NT5 — orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; NT50 — orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; NT500 — orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; NT2000 — orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; CM5 — orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; CM50 — orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; CM500 — orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; CM2000 — orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

oxidative stress-stimulated inflammatory responses observed with DS groups can initiate programmed apoptosis via the activation of the NFκB-dependent p53 signaling pathway and thereby, upregulated p53 gene expression which triggers loss and death of striatal dopamine-producing cells (Yan et al., 2014). Elevated IL-1β and TNF-α concentrations can also directly induce neuronal apoptosis by employing adaptive and innate immune cells, and excessive glial hyperactivation (Tjalkens et al., 2017). This cascade coupled with the obtained results for the stimulation of NFκB and p53 gene expression of experimental rats' striatum at the high doses of DS may indicate addiction-induced neurodegeneration and cell death pathway of the DS alkaloid extracts.

Purinergic neurotransmitters have also been implicated in the etiology of drug addiction and neurotoxicity (Rozisky et al., 2010; Wu and Li, 2020). E-NTPDases hydrolyze ATP and ADP while AMP is hydrolyzed 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

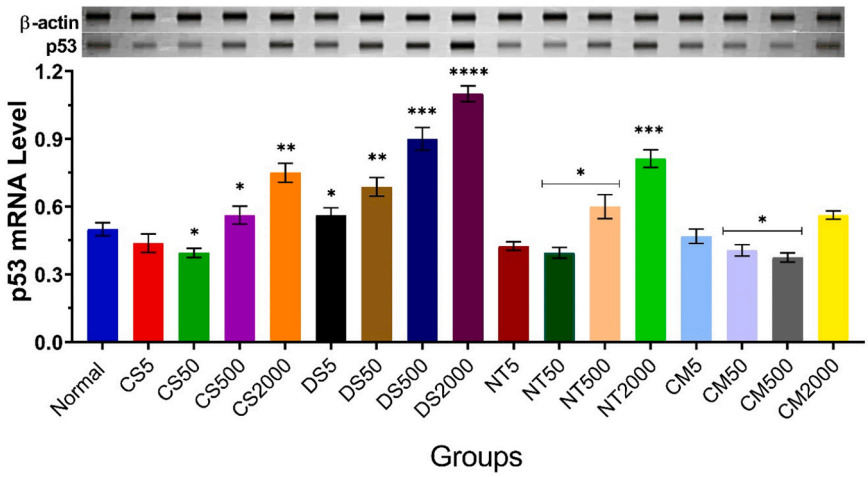

Fig. 19. Effects of CS, DS, NT, and CM on p53 mRNA expression. Results are expressed as mean ± SEM (n = 6). *p < 0.05, * *p < 0.01, * **p < 0.001, * ** *p < 0.0001 compared with the normal control group. CS5 — orally administered alkaloid extracts of *C. sativa* at 5 mg/kg bwt; CS50 — orally administered alkaloid extracts of *C. sativa* at 50 mg/kg bwt; CS500 — orally administered alkaloid extracts of *C. sativa* at 500 mg/kg bwt; CS2000 — orally administered alkaloid extracts of *C. sativa* at 2000 mg/kg bwt; DS5—orally administered alkaloid extracts of *D. stramonium* at 5 mg/kg bwt; DS50 — orally administered alkaloid extracts of *D. stramonium* at 50 mg/kg bwt; DS500 — orally administered alkaloid extracts of *D. stramonium* at 500 mg/kg bwt; DS2000 — orally administered alkaloid extracts of *D. stramonium* at 2000 mg/kg bwt; NT5 — orally administered alkaloid extracts of *N. tabacum* at 5 mg/kg bwt; NT50 — orally administered alkaloid extracts of *N. tabacum* at 50 mg/kg bwt; NT500 — orally administered alkaloid extracts of *N. tabacum* at 500 mg/kg bwt; NT2000 — orally administered alkaloid extracts of *N. tabacum* at 2000 mg/kg bwt; CM5 — orally administered alkaloid extracts of *C. papaya* at 5 mg/kg bwt; CM50 — orally administered alkaloid extracts of *C. papaya* at 50 mg/kg bwt; CM500 — orally administered alkaloid extracts of *C. papaya* at 500 mg/kg bwt; CM2000 — orally administered alkaloid extracts of *C. papaya* at 2000 mg/kg bwt.

via specific G-protein-coupled P1 purinoceptors, $A_{2A}R$, and A_{2A}-D_2 receptor-receptor interactions in dorsal striatum to mediate addiction and habitual substance-seeking behaviors (Pintsuk et al., 2016; Burnstock, 2017). However, stimulation of the striatal $A_{2A}R$ has been implicated in mediating fear, and elevating glutamate induced-excitotoxicity and IL-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; Ademosun et al., 2022). Contra-wise, the inhibition of ecto-5'-nucleotidase and E-NTPDase activities by DS has 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 (Ademiluyi et al., 2016; Fasakin et al., 2021). Considerably, CS, DS, NT, and CM can trigger addiction and neurotoxicity via the purinergic neurotransmission pathway.

Comparatively, DS groups showed heightened inflammatory, apoptotic, and enzyme and neurotransmitter imbalances. In contrast, the differences in other groups were less significant, which may be indicative that the multifaceted disruptions experienced by the DS groups were to a lesser extent in different groups. Although DS contains tropane alkaloids such as scopolamine, atropine, and hyoscyamine, which have psychoactive properties, it interacts with the central nervous system, particularly by blocking acetylcholine receptors, leading to alterations in neurotransmitter levels (Fasakin et al., 2022a). The DS alkaloids lead to a wide array of systemic imbalances, including disruptions in neurotransmission, enzyme regulation, and immune function, which could explain why these imbalances are more pronounced in these groups compared to others (Ademiluyi et al., 2016; Fasakin et al., 2021). Additionally, the body interprets tropane alkaloids as toxins, leading to the release of pro-inflammatory cytokines. Inflammation could also be a consequence of oxidative stress, which is often induced by tropane alkaloid exposure, leading to an overactive inflammatory response (Lawal et al., 2019; Fasakin et al., 2022a). The toxicity of tropane alkaloids can also induce cellular stress, mitochondrial dysfunction, and oxidative damage, which in turn may trigger apoptosis (Gjorgieva Ackova et al., 2023). Furthermore, observed enzyme imbalances, oxidative damage, and apoptosis were lesser in the CS, NT, and CM groups due to the plants containing medicinal alkaloids that can compensate for the neurotoxic ones, e.g., cannabidiol compensates for Δ-9-tetrahydrocannabinol neurotoxicity (Fasakin et al., 2022a, 2022b).

5. Conclusion

The result of this study showed a strong link 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 CS > NT > DS > CM in addictiveness but ranked DS > NT > CS > CM for toxicological potentials. Notably, the toxicological effects of the psychoactive plants' alkaloids are mostly expressed at high doses. However, further studies are recommended to determine how the participation of the discussed brain systems can be employed during the treatment and management of addiction and neurotoxicity.

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Ethical statement

Ethical approval was obtained from the Animal Ethical Committee, Centre for Research and Development (CERAD) of the Federal University of Technology, Akure with the ethical number FUTA/ETH/2020/016 in December 2020.

CRediT authorship contribution statement

Ganiyu Oboh: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Formal analysis, Data curation,

Conceptualization. **Ayokunle O. Ademosun:** Writing – review & editing, Writing – original draft, Supervision, Software, Methodology, Investigation, Data curation, Conceptualization. **Olamide Wilson Fasakin:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

All authors of this study declare that there is no potential 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.

Data Availability Statement

No additional data/information is available for this paper.

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