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ORIGINAL ARTICLE

Neuromodulatory evaluation of commonly abused plants ex vivo: a comparative study

Olamide W. Fasakin[1] · Ganiyu Oboh[1] · Ayokunle Olubode Ademosun[1]

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Abstract

Nicotiana tabacum, *Datura stramonium*, and *Carica papaya* are plants that are on the high trend as substitutes to conventional psychoactive substances due to their legality and the difference in the experiences they offer. The present study was aimed at comparing the neuromodulatory and toxicological potentials of the afore-named plants to an illicit psychoactive plant, *Cannabis sativa*. Consequently, the effects of the alkaloid extract of the plants were evaluated on critical neuronal enzymes of the monoaminergic, cholinergic, and purinergic (sodium/potassium adenosine triphosphatase (Na^+/K^+-ATPase), ecto-5'-nucleotidase [eNTDase], and ecto-nucleoside triphosphate diphosphohydrolase [E-NTPDase]) systems of neurotransmission, reactive oxygen species (ROS) production, and lipid peroxidation in rat brain tissue homogenate ex vivo. Plants' alkaloids were prepared by solvent extraction method. Results revealed that the extracts inhibited the enzymes in a concentration-dependent manner. However, *C. sativa* had the highest inhibition of monoamine oxidase (MAO), eNTDase, and E-NTPDase activities, while *D. stramonium* had the highest cholinesterase and Na^+/K^+-ATPase activity inhibition, and ROS production and lipid peroxidation. In conclusion, *D. stramonium* altered critical neuronal enzymes significantly more than the illicit plant of abuse, while *Nicotiana tabacum* showed no significant difference in comparison to *C. sativa.* Therefore, the use of these plants as drugs should be discouraged.

Keywords *Cannabis sativa* · *Nicotiana tabacum* · *Datura stramonium* · *Carica papaya* · Monoaminergic · Cholinergic · Purinergic

Introduction

As old as human history, plant-based drugs have their usage in religious rites and medicines by various ancient traditions. These plants are commonly referred to as psychoactive plants due to their ability to affect the mind or alter the state of consciousness, by directly or indirectly modulating neurotransmitter systems, such as the dopaminergic, cholinergic, purinergic, serotonergic, noradrenalinegic, and Y-butyric; pathways; and their receptors (Cunha-Oliveira et al. 2008). Toxicity and dependency on these plants have been associated with alkaloids which have the potentials of inducing hallucinogenic, stimulatory, or euphoric effects (Graziano et al. 2017). However, recorded abuse was dated to the 19th century during the Ching Dynasty of China resulting in the enactment of laws during the 1961 Single Convention on Narcotic Drugs with further reviews during the Conventions on Psychotropic substances in 1971 and Illicit Traffic in Narcotic Drugs and Psychotropic Substances in 1988 (Feng et al. 2017). Recent data showed that abuse of psychoactive plants is not limited to *Cannabis sativa* (marijuana), *Papayer somniferum* (opium poppy), *Erythroxylon coca* (cocaine), magic mushrooms, etc., but that many other plant-based drugs which pose threat to public health are elusive. They were thus defined by UNODC (United Nation Office on Drugs and Crime) as new psychoactive substances (NPS) of plant origin or new psychoactive plants (NPP), of which about 20 were reported among the total 230 psychoactive substances reported in-between January 2005 and December 2012 by European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (Ujváry 2014). These new psychoactive plants have thus served as alternatives to the illicit plant-based drugs due to the false perception of them being legal and organic, thereby safe. As much as the surge prevalence

✉ Ganiyu Oboh
goboh@futa.edu.ng

[1] Functional Foods, Nutraceuticals and Phytomedicine Unit, Department of Biochemistry, Federal University of Technology, P.M.B. 704, Akure 340001, Nigeria

and abuse of these plants, data on toxicological, pharmacological, and clinical effects are limited. Putting into consideration a large number of plants of abuse, there is, therefore, a need for techniques that capture multiple mechanisms of action of the plants in comparison with the conventional psychoactive substances while screening ex vivo. Commonly abused of these new psychoactive plants in Nigeria are *N. tabacum*, *D. stramonium*, and *C. papaya* (male and female). Most plant extract IC_{50} values usually occur close to the estimated brain concentrations following recreational abuse of these plants, indicating their efficiency in ex vivo approach for toxicological and classification screening. This study aims to compare the neuromodulatory and toxicological potentials of these plants of abuse to *C. sativa* L, an illicit psychoactive plant.

Materials and methods

Materials

Drugs and chemicals

ATPase, ADPase, AMPase, adenosine, acetic acid, methanol, acetylthiocholine, and butyrylthiocholine iodide were purchased from Sigma (St Louis, MO, USA) and Merck (Darmstadt, Germany). All other chemicals used in the present study were of analytical grade.

Methods

Sample collection and preparation

Fresh leaves of *C. sativa*, *N. tabacum*, and *C. papaya* (male and female), and seeds of *Datura stramonium* were obtained in Akure South Local Government of Ondo State, Nigeria. The plants were identified and authenticated at the Centre for Research and Development, Federal University of Technology, Akure, Nigeria. The leaves and seeds were carefully cleansed of stalks, debris, and other unwanted materials, sundry, and blend into powder using an electric blender.

Preparation of alkaloid extracts

Alkaloid extracts of *C. sativa*, *N. tabacum*, *D. stramonium*, and *C. papaya* (male and female) were prepared using the method of Harborne (1998), as modified by Ademiluyi et al. (2016b). Typically, 100 g of powdered samples were defatted for 24 h with n-hexane. A total of 200 mL of 10% acetic acid in ethanol were thereafter 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. Concentrated ammonium hydroxide dropwise 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, which were stored frozen for further analysis at 4 °C.

Animal handling

Twenty (20) male albino Wistar rats weighing between 210 and 212 g were obtained from the Animal House of the Department of Biochemistry, Federal University of Technology, Akure, Ondo State, Nigeria. Their handling and use were approved by 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. The animals were kept in plastic cages and housed at room temperature (25–27 °C), relative humidity (60–70%), and controlled light cycle (12-h dark/12-h light) for 2 weeks with free access to commercial rat chow and water ad libitum before use.

Preparation of tissue homogenate

The rats were anesthetized with isofluorane before rapid decapitation, after which the whole brain tissue was rapidly removed, rinsed with cold saline to remove blood stains. Thereafter, the brain was weighed and then homogenized with Tris-HCl buffer (1/5 w/v) (pH 7.4) with 10-up-and-down strokes at about rev/min in a Teflon glass (Mexxcare, mc14 362, Aayu-shi Design Pvt. Ltd. India) homogenizer (Adefegha et al. 2015). Afterward, the homogenate was centrifuged at 3000 rpm for 10 min at 4 °C using a refrigerated centrifuge (KX3400C, KENXIN Intl. Co., Hong Kong). The supernatant was obtained and used for biochemical assays, while the pallet was discarded.

Monoamine oxidase activity assay

The inhibitory potentials of *C. sativa*, *N. tabacum*, *D. stramonium*, and *C. papaya* (male and female) alkaloid extracts on MAO activity were assayed according to the method of Turski et al. (1973) with slight modification. To the varying concentrations of the alkaloid extracts (0–25 µg/mL) were added 150 µL of 0.025 M phosphate buffer (pH 7.0), 12.5 mM semicarbazide, 10 mM benzylamine, and 100 µL of brain homogenate, glacial acetic acid was then added to the mixture after 30 min and incubated in boiling water for 3 min and then centrifuged. One (1) ml of the supernatant was mixed with 1.25 ml of benzene and 1 ml of 2, 4-DNPH, and incubated at room temperature for 10 min. One (1) ml of the benzene layer was then mixed with 1 ml of 0.1 N NaOH.

The alkaline layer was decanted and incubated for 10 min at 80 °C, the absorbance read at 450 nm. The MAO inhibitory potential of the samples was calculated as stated below;

$$\%\ \text{MAO Inhibition} = \frac{Abs_{ref} - Abs_{sam}}{Abs_{ref}} \text{ x } 100$$

where: Abs_{ref} = absorbance of reference
Abs_{sam} = absorbance of sample

Cholinesterase activity assay

The inhibitory potentials of alkaloid extract from *C. sativa*, *N. tabacum*, *D. stramonium*, and *C. papaya* (male and female) on cholinesterase (acetylcholinesterase [AChE] and butyrylcholinesterase [BChE]) activities were assayed according to the method of Perry et al. (2000). To the varying concentrations of the alkaloid extracts (0–15 µg/mL) were added 30 µL of 10 mM 5, 5′-dithio-bis (2-nitrobenzoic) acid (DTNB), 30 µL of brain homogenate in 0.1 M phosphate buffer (pH 8.0), and 0.1 M phosphate buffer (pH 8.0). The reaction mixture was incubated at 25 °C for 20 min, after which acetylthiocholine iodide (for AChE) or butyrylthiocholine iodide (for BChE) was added as substrate, the absorbance read at 450 nm and the AChE/BChE inhibitory potential of the samples was calculated as stated below:

$$\%\ \text{AChE/BChE Inhibition} = \frac{Abs_{ref} - Abs_{sam}}{Abs_{ref}} \text{ x } 100$$

Where: Abs_{ref} = absorbance of reference
Abs_{sam} = Absorbance of Sample

Ecto-nucleoside triphosphate diphosphohydrolase activity assay

The inhibitory potentials of *C. sativa*, *N. tabacum*, *D. stramonium*, and *C. papaya* (male and female) alkaloid extracts on ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase) (using ATP and ADP as substrate) activity were assayed (Schetinger et al. 2007). A total of 150 µL of the substrate (ATP or ADP) was added to the varying concentrations of the alkaloid extracts (0–25 µg/mL), and 100 µL of $CaCl_2$ and Tris-HCl buffer (pH 7.4) to make a total volume of 350 µL. The reaction mixture was incubated for 10 min at 37 °C, after which 150 µL of brain homogenate, incubated for a further 20 min, and 500 µL of 10% of trichloroacetic acid was then added. The reaction mixture was allowed to chill on ice for 10 min, and the supernatant was used to assay for released inorganic phosphate (Pi) according to the method of Fiske and Subbarow (1925). The ATPase/ADPase activity was calculated as stated below and expressed as percentage ATP/ADP inhibition:

$$\%\ \text{ATP/ADP Inhibition} = \frac{Abs_{ref} - Abs_{sam}}{Abs_{ref}} \times 100$$

Where: Abs_{ref} = absorbance of reference
Abs_{sam} = absorbance of sample

Ecto-5′ nucleotidase assay

The inhibitory potentials of *C. sativa*, *N. tabacum*, *D. stramonium*, and *C papaya* (male and female) alkaloid extracts on ecto-5-nucleotidase (eNTDase) activity were assayed according to the method of Heymann et al. (1984). To the varying concentrations of the alkaloid extracts (0–25 µg/mL) were added 150 µL AMP, 100 µL of $MgCl_2$, and Tris-HCl buffer (pH 7.4) to make a total volume of 350 µL. The reaction mixture was incubated for 10 min at 37 °C, after which 150 µL of brain homogenate, incubated for a further 20 min, and 500 µL of 10% of trichloroacetic acid was then added. The reaction mixture was allowed to chill on ice for 10 min, and the supernatant was used to assay for released inorganic phosphate (Pi) according to the method of Fiske and Subbarow (1925). The inhibition of ecto-5-nucleotidase was calculated as stated below:

$$\%\text{AMPInhibition} = \frac{Abs_{ref} - Abs_{sam}}{Abs_{ref}} \times 100$$

Where: Abs_{ref} = absorbance of reference
Abs_{sam} = absorbance of sample

Na^+/K^+-ATPase activity assay

The inhibitory potentials of *C. sativa*, *N. tabacum*, *D. stramonium*, and *C. papaya* (male and female) alkaloid extracts on Na+/K+-ATPase activities were assayed according to the method of Wyse et al. (2000). A total of 100 µL Na^+/K^+-ATPase substrate (Ouabain), buffer (pH 7.4) (containing 120 mM Tris-HCl, 0.4 mM EDTA, 200 mM NaCl, 20 mM KCl, and 24 mM $MgCl_2$), 50 µL of homogenate, and 50 µL of 1 MM ouabain were added to the varying concentrations of the alkaloid extracts (0–15 µg/mL) to make a total volume of 200 µL. The reaction was initiated by adding 50 µL of adenosine triphosphate (ATP), incubated at 37 °C for 30 min, and terminated by adding 50 µL of 10% trichloroacetic acid (TCA). The reaction mixture was allowed to chill on ice for 10 min, and the supernatant was used to assay for released inorganic phosphate (Pi) according to the method of Fiske and Subbarow (1925). The percentage Na^+/K^+-ATPase inhibition was calculated as stated below:

$$\%\ Na^{+}/K^{+}-\ \text{ATPase Inhibition} = \frac{Abs_{ref} - Abs_{sam}}{Abs_{ref}} \text{ x } 100$$

Where: Abs_{ref} = absorbance of reference
Abs_{sam} = absorbance of sample

Reactive oxygen species assay

The formation of reactive oxygen species potentials of *C. sativa*, *N. tabacum*, *D. stramonium*, and *C. papaya* (male and female) were estimated as H_2O_2 equivalent according to the method of Oboh et al. (2018) using the n-n-diethyl-para-phenylenediamine (DEPPD) reagent. -n-Diethyl-para-phenylenediamine (DEPPD) reagent was added to the varying concentrations of the alkaloid extracts (0–15 µg/mL), and the mixture was incubated for 5 min at 37 °C. Absorbance was measured using a spectrophotometer at 505 nm, while the standard calibration curve of H_2O_2 was used to quantify reactive oxygen species levels and expressed in unit/mg protein, wherein 1 unit equals 1 mg H_20_2/L.

Thiobarbituric acid-reactive substance formation assay

The formation of thiobarbituric acid-reactive substances (TBARS) by *C. sativa*, *N. tabacum*, *D. stramonium*, and *C papaya* (male and female) were assayed according to the method of Ohkawa et al. (1979) with slight modification. A total of 50 µL of brain homogenate, 300 µL of 8.1% sodium dodecyl sulfate (SDS), 500 µL HCl/acetic acid (pH 3.4), and 500 µL of thiobarbituric acid (TBA) were added to the varying concentrations of the alkaloid extracts (0–25 µg/mL), and the mixture was incubated for 1 h at 100 °C. The formed TBARS was quantified using a spectrophotometer at 532 nm and calculated as TBARS produced.

Data analysis

All data were analyzed and expressed as values representing triplicate readings (mean ± standard deviation), while the level of significant difference ($P \leq 0.05$) was by one-way analysis of variance (ANOVA) using Duncan multiple test. IC50 values (effective concentration that caused 50% inhibition) were calculated with non-linear regression analysis, while the statistical analyses were carried out via version 5 of the GraphPad Prism.

Results

Typically, Fig. 1 shows the effects of the alkaloid extracts of C. *sativa*, *N. tabacum*, *D. stramonium*, and *C. papaya* (male and female) on the activity of MAO. The alkaloid extracts inhibited MAO activity in a concentration dependent manner. As revealed by IC_{50} value, *C. sativa* had the highest (12.34 ± 0.27 µg/ml) value closely followed by that of *Nicotiana tabacum* (12.99 ± 0.31 µg/ml), *D. stramonium* (17.47 ± 0.0.35 µg/ml), *C. papaya* (20.76 ± 0.42 µg/ml), while *C. papaya* female (28.96 ± 0.58 µg/ml) has the least when the effects of alkaloid extracts from *C. sativa*, *N. tabacum*, *D. stramonium*, and *C. papaya* (male and female) were compared on ex vivo activity of MAO. More so, as shown in Figs. 2 and 3, it was observed that alkaloid extract from *C. sativa*, *N. tabacum*, *D. stramonium*, and *C. papaya* (male and female) inhibited the cholinesterase (AChE and BChE) activity in a concentration dependent manner. Finding from this study revealed that AChE and BChE activity was significantly ($P < 0.05$) inhibited by alkaloid extracts from *C. sativa*, *N. tabacum*, *D. stramonium*, and *C. papaya* (male and female). Nevertheless, as revealed by IC_{50} value, *D. stramonium* had highest ((AChE = 5.48±0.19 µg/ml; BChE = 12.10 ± 0.25 µg/ml)) inhibitory effect, while *C.*

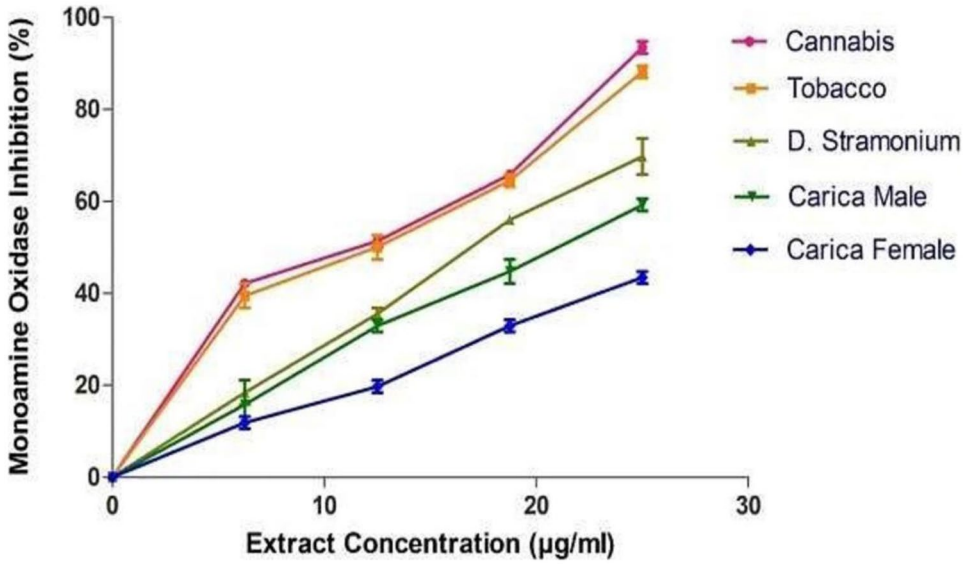

Fig. 1 The percentage inhibitory effects of alkaloid extracts of *Cannabis sativa*, *Nicotiana tabacum*, *Datura stramonium*, and *Carica papaya* (male and female) on the activity of monoamine oxidase (MAO). Values represent triplicate readings (mean ± standard deviation)

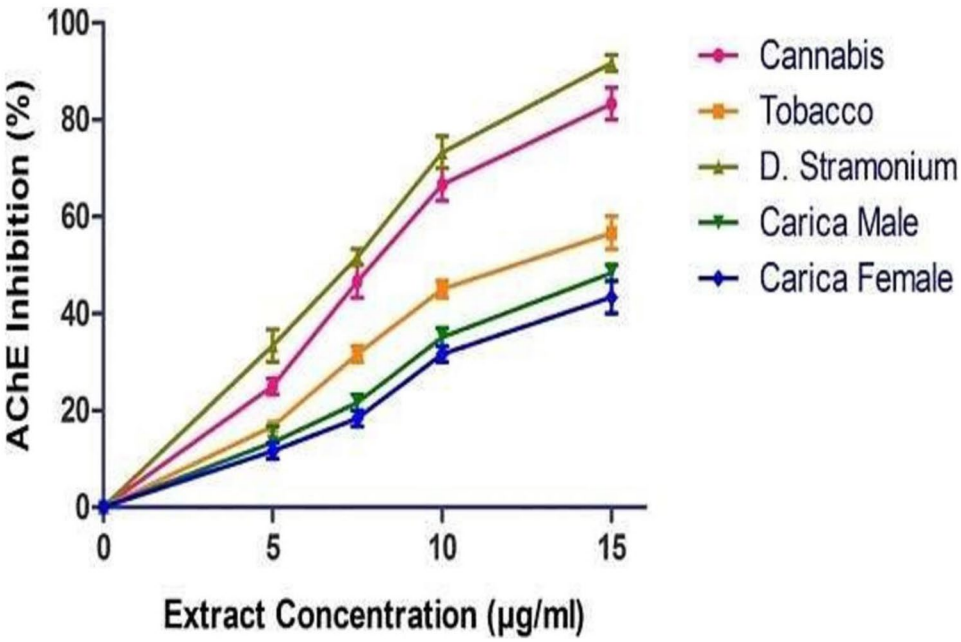

Fig. 2 The percentage inhibitory effects of alkaloid extracts of *Cannabis sativa*, *Nicotiana tabacum*, *Datura stramonium*, and *Carica papaya* (male and female) on the activity of acetylcholinesterase (AChE). Values represent triplicate readings (mean ± standard deviation)

sativa ((AChE = 6.60 ± 0.20 µg/ml; BChE = 14.48 ± 0.29 µg/ml)), *N. tabacum* ((AChE = 11.30 ± 0.29 µg/ml; BChE = 21.89 ± 0.42 µg/ml)), *C. papaya* male ((AChE = 14.28 ± 0.33 µg/ml; BChE = 34.40 ± 0.67 µg/ml)), while *C. papaya* female ((AChE = 16.19 ± 0.37 µg/ml; BChE = 40.93 ± 0.80 µg/ml)) had the least inhibitory effect on ex vivo activity of AChE and BChE. Figures 4 and 5, respectively, depict the effect of alkaloid extract from *C. sativa*, *N. tabacum*, *D. stramonium*, and *C. papaya* (male and female) on ex vivo activity of ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase and ADPase). The results revealed that alkaloid extracts exhibited dose response inhibitory effect on ex vivo activity ecto-nucleoside triphosphate diphosphohydrolase with *C. sativa* ((ATPase = 13.51 ± 0.30 µg/ml; ADPase = 18.19 ± 0.36 µg/ml)) had highest inhibitory effect followed by *D. stramonium* ((ATPase = 14.23 ± 0.32 µg/ml; ADPase = 18.73 ± 0.37 µg/ml)), *N. tabacum* ((ATPase = 17.12 ± 0.35 µg/ml; ADPase = 24.29 ± 0.48 µg/ml)), *C. papaya* male ((ATPase = 22.64 ± 0.43 µg/ml; ADPase = 50.49 ± 1.02 µg/ml)), while *C. papaya* female ((ATPase = 25.27 ± 0.47 µg/ml; and ADPase = 53.79 ± 1.08 µg/ml)) had the least when their effects were compared. The present study also observed the same trend for ecto-5-nucleotidase (eNTDase) activity inhibition by the plant extracts (Fig. 6). *C. sativa* had the highest modulatory effect (AMPase = 14.91 ± 0.34 µg/ml), followed by *D. stramonium* (AMPase = 15.11 ± 0.34 µg/ml), then *N. tabacum* (AMPase = 18.39 ± 0.43 µg/ml) and *C. papaya* male (AMPase = 20.45 ±

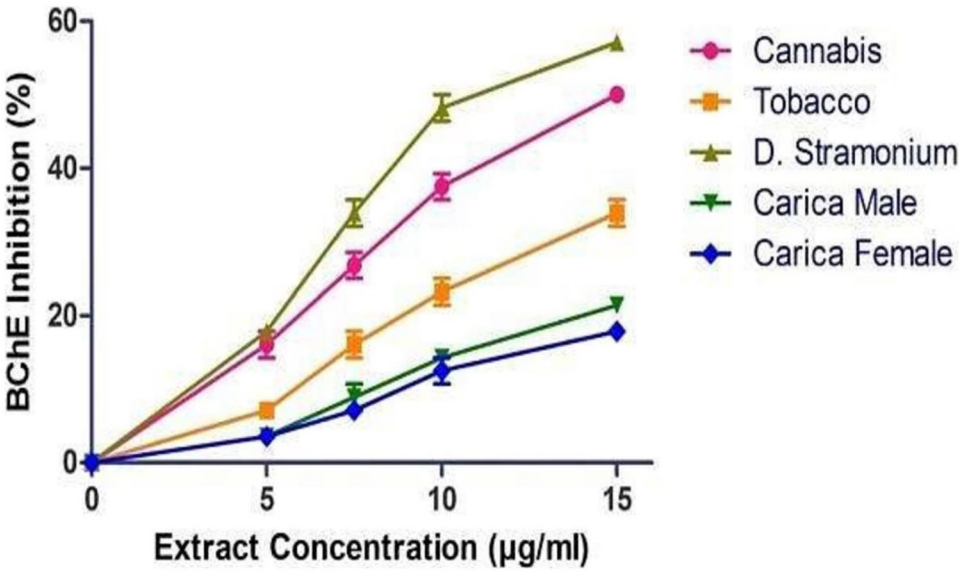

Fig. 3 The percentage inhibitory effects of alkaloid extracts of *Cannabis sativa*, *Nicotiana tabacum*, *Datura stramonium*, and *Carica papaya* (male and female) on the activity of butyrylcholinesterase (BChE). Values represent triplicate readings (mean ± standard deviation)

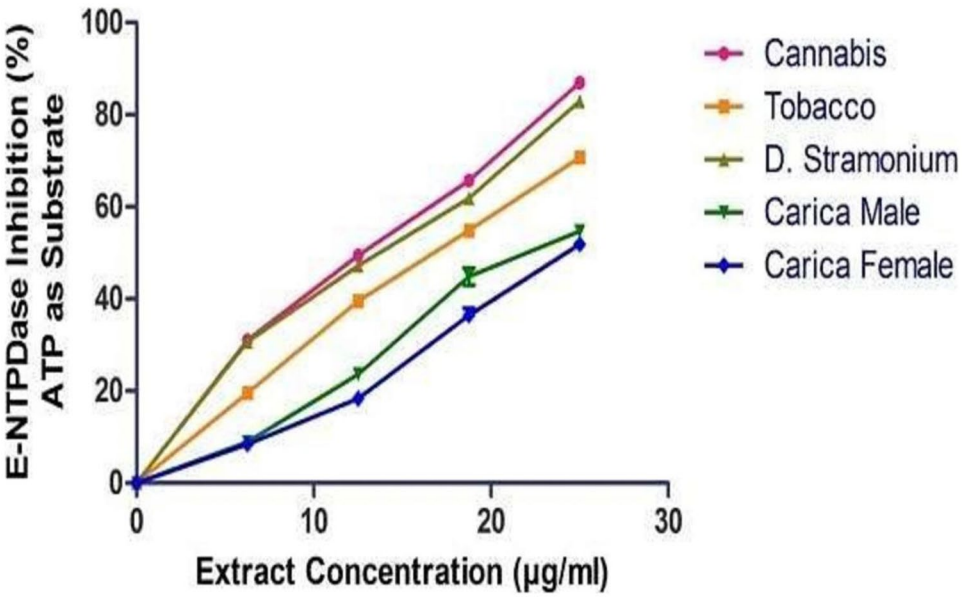

Fig. 4 The percentage inhibitory effects of alkaloid extracts of *Cannabis sativa*, *Nicotiana tabacum*, *Datura stramonium*, and *Carica papaya* (male and female) on the activity of E-NTPDase activity using ATP as substrate. Values represent triplicate readings (mean ± standard deviation)

0.44 µg/ml) and finally *C. papaya* female (AMPase = 22.77 ± 0.50 µg/ml). Also, Na+/K+-ATPase activity inhibition was also observed to be affected by the alkaloid extracts in a concentration-dependent manner (Fig. 7). Considering the effects of the extracts using 50% modulatory inhibitory effect on Na+/K+-ATPase activity, *D. stramonium* (Na+/K+-ATPase = 8.36 ± 0.19 µg/ml) had higher inhibitory effect than even *C. sativa* (Na+/K+-ATPase = 9.44 ± 0.20 µg/ml), an illicit psychoactive substance. Meanwhile, *N. tabacum* Na+/K+-ATPase = 11.21 ± 0.23 µg/ml), *Carica papaya* male (Na+/K+-ATPase = 19.89 ± 0.38 µg/ml), and *C. papaya* female (Na+/K+-ATPase = 21.40 ± 0.42 µg/ml) values were also observed. Finally, the present study investigated the effect of the alkaloid extracts on H_2O_2 production and lipid peroxidation (Figs. 8 and 9). H_2O_2 production and lipid peroxidation levels were significantly ($P < 0.05$)

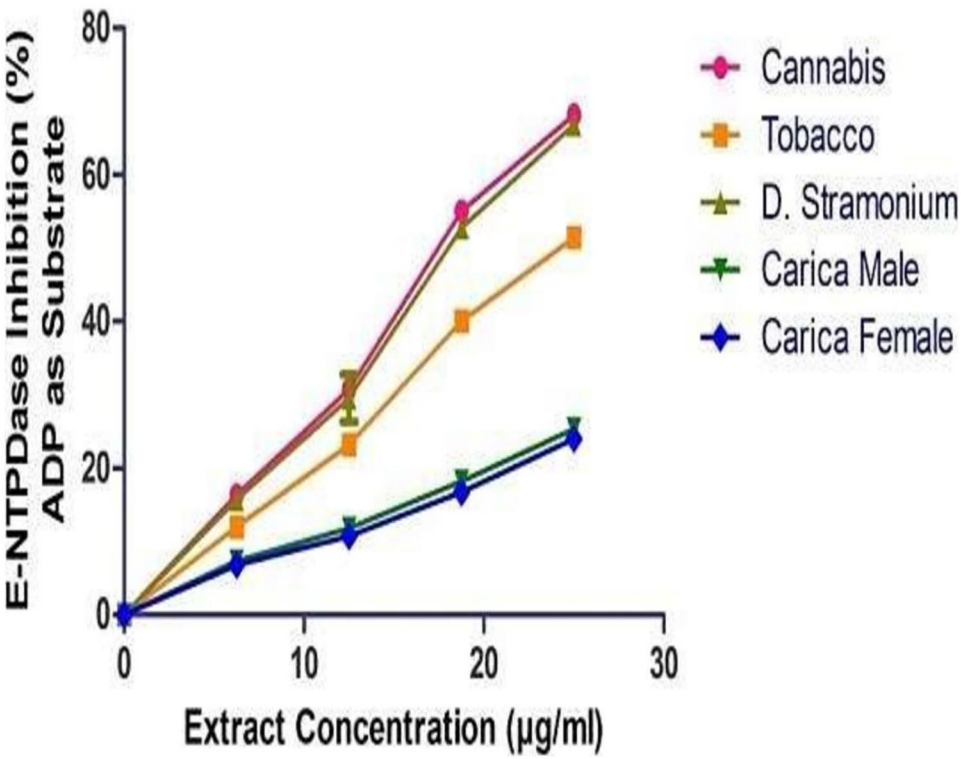

Fig. 5 The percentage inhibitory effects of alkaloid extracts of *Cannabis sativa*, *Nicotiana tabacum*, *Datura stramonium*, and *Carica papaya* (male and female) on the activity of E-NTPDase activity using ADP as substrate. Values represent triplicate readings (mean ± standard deviation)

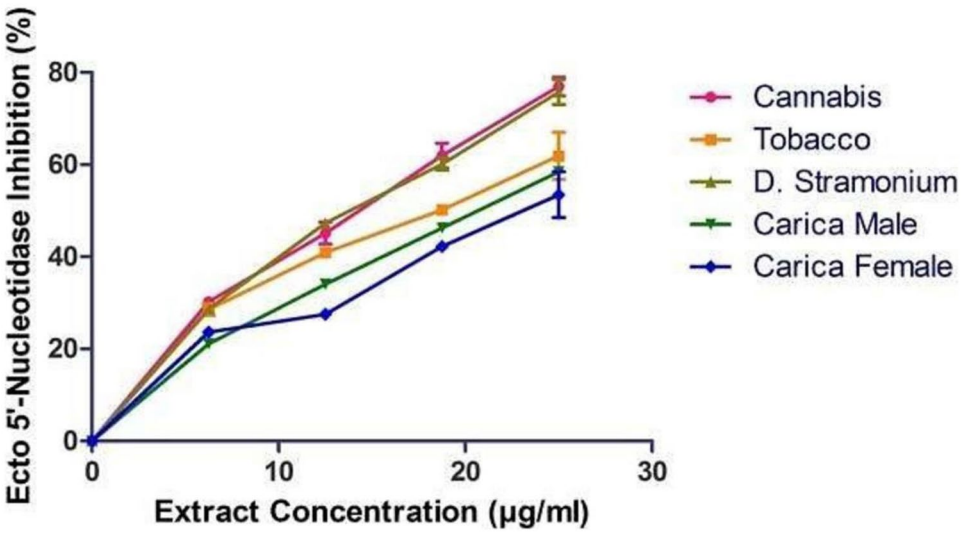

Fig. 6 The percentage inhibitory effects of alkaloid extracts of *Cannabis sativa*, *Nicotiana tabacum*, *Datura stramonium*, and *Carica papaya* (male and female) on the activity of E-NTDase activity using AMP as substrate. Values represent triplicate readings (mean ± standard deviation)

elevated in the present study with *D. stramonium*, having the highest values of 8.18 ± 0.15 µg/ml and 13.77 ± 0.25 µg/ml, respectively. Our control, *C. sativa* also caused H_2O_2 production and lipid peroxidation levels 8.94 ± 0.16 µg/ml and 14.97 ± 0.28 µg/ml, respectively, which is not significantly different from that observed of *D. stramonium*. Furthermore, ROS production and lipid peroxidation levels of *N. tabacum* (9.71 ± 0.18 µg/ml, and 18.57 ± 0.34 µg/ml), *C. papaya* male (29.48 ± 0.58 µg/ml, and 39.98 ± 0.73 µg/ml) and *C. papaya* female (34.55 ± 0.67 µg/ml, and 48.95 ± 0.91 µg/ml) were also observed.

Discussion

A plant-based psychoactive substance is a substance, which has its primary effect directed at the state of consciousness through the direct or indirect modulation of neurotransmitter systems, such as the dopaminergic, cholinergic, and purinergic systems and their receptors (Cunha-Oliveira et al. 2008). The present study observed that *C. sativa* had the highest MAO inhibition of which can be due to the presence of delta-9-tetrahydrocannabinol (THC), its active psychoactive constituent as observed by (Baggio et al. 2014), though less potent compared to that observed with iproniazid, a standard non-selective monoamine oxidase inhibitor (IC_{50} = 0.72 µg/mL) by Zhi et al. (2016). Close to the illicit substance is *N. tabacum* which can be relatively due to the presence of its active constituents—nicotine and beta-carboline alkaloids (harman and nor-harman), which are present in tobacco smoke (Herraiz and Chaparro 2005). Typically, these two plant-based psychoactive substances have always been on the top chart of the most abused substances (World Drug Report 2015) with their high level of addiction and high range of acceptance by users cutting across the global world (Hasin et al. 2013; World Drug Report 2015). More so, a

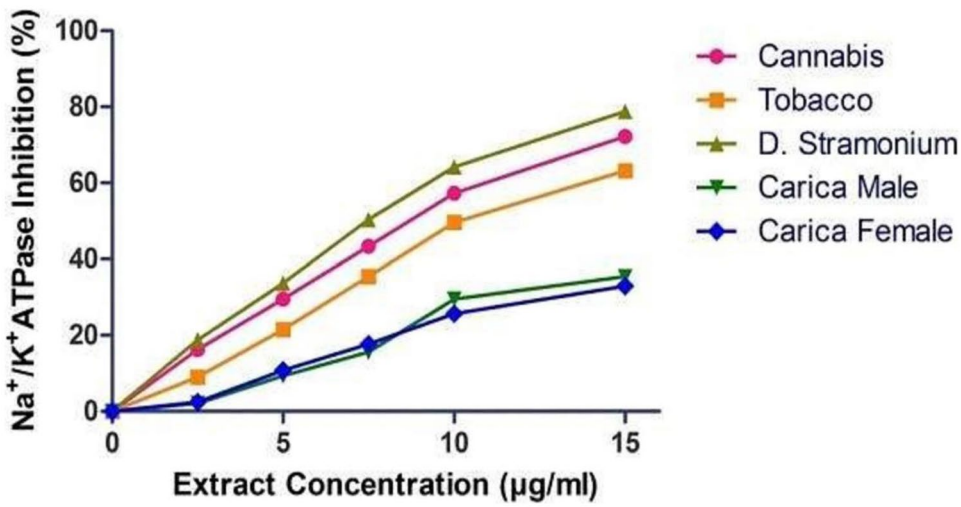

Fig. 7 The percentage inhibitory effects of alkaloid extracts of *Cannabis sativa*, *Nicotiana tabacum*, *Datura stramonium*, and *Carica papaya* (male and female) on the activity of Na^+/K^+-ATPase activity. Values represent triplicate readings (mean ± standard deviation)

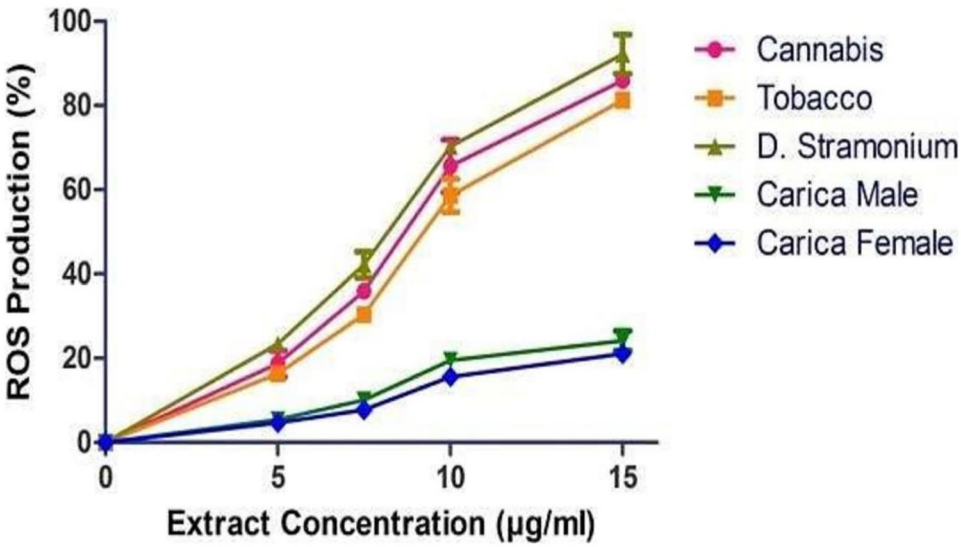

Fig. 8 The percentage ROS production levels of alkaloid extracts of *Cannabis sativa*, *Nicotiana tabacum*, *Datura stramonium*, and *Carica papaya* (male and female). Values represent triplicate readings (mean ± standard deviation)

considerable inhibition of MAO was observed in *D. stramonium*, though its high level of toxicity and lethal effects as observed by Ademiluyi et al. (2016a, b) and Ogunmoyole et al. (2019) can be the underline factor marring its addictiveness and dependence. Meanwhile, the level of amino acids tyrosine, and phenylalanine, and tryptophan, the precursors of norepinephrine and serotonin, respectively, in *C. papaya* (male and female) might be the underline factor of their MAO inhibition as observed by Parle (2011). Furthermore, the higher IC_{50} value observed in the male *C. papaya* compares to that of the female *Carica papaya* might be the underline factor for its preference over the female counterpart by abusers as observed in folklore.

As much as the neurological importance of the activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are, their high inhibition as observed in the present study can ultimately result in excess accumulation of the choline at the synaptic cleft in living organisms when unchecked, causing overstimulation of the cholinergic and postsynaptic neurons and resultantly leading to neuronal death and/or even the organism's death (Ademiluyi et al. 2016b; Rodrigues et al. 2011). Notably, the present study observed that *D. stramonium* had higher AChE and BChE inhibitory values compared to *C. sativa*, an illicit psychoactive substance. The high level of cholinesterase activity inhibition by *D. stramonium* correlates with previous studies that associated the high inhibition with the presence of alkaloids such as atropine, hyoscyamine, and scopolamine. Furthermore, *N. tabacum* and *C. papaya* (male and female) were also observed to pose a significant inhibitory effect, with *C. papaya* female exhibiting a lesser potency compared to the male species.

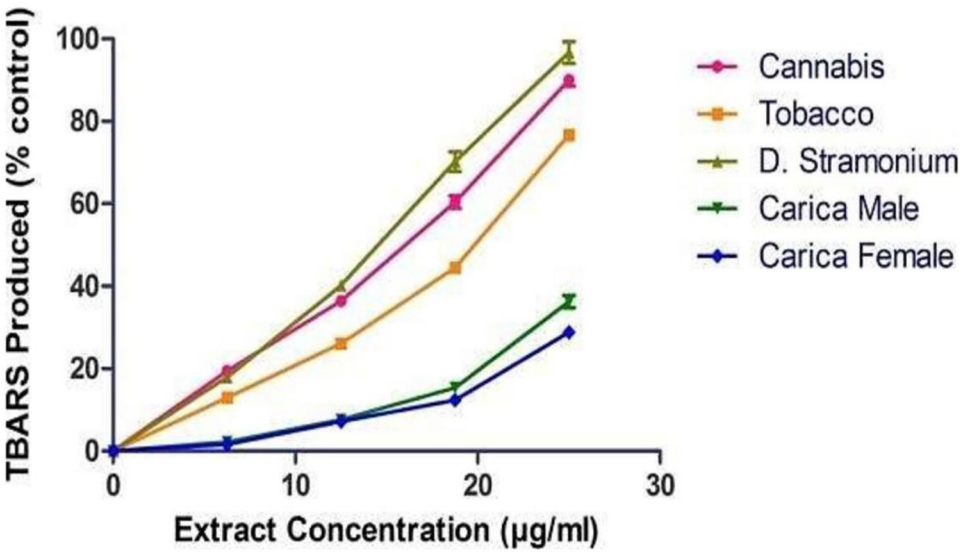

Fig. 9 The percentage lipid peroxidation levels of alkaloid extracts of *Cannabis sativa*, *Nicotiana tabacum*, *Datura stramonium*, and *Carica papaya* (male and female). Values represent triplicate readings (mean ± standard deviation)

The inhibition of E-NTPDase activities as observed in the present study could lead to impairments of the hydrolysis of neuronal ATP resulting in excessive stimulation of the P2 purinergic receptors and thereby a potential aberration of the purinergic neurotransmission system. This result is in line with previous studies that suggested that alkaloid's E-NTPDase inhibitory abilities are associated with their neurotoxicity potentials (Colović et al. 2015; Senger et al. 2005). In addition, the present study also observed inhibition of ecto 5-nucleotidase by *C. sativa*, *D. stramonium*, *Nicotiana tabacum*, and *Carica papaya* alkaloid extracts which coupled with that observed on the ATPase and ADPase activities could result in excess depletion of adenosine levels extracellularly. Meanwhile, excess depletion of adenosine, the neuromodulator implicated in memory and synaptic plasticity formation, has been implicated with adrenergic neurotransmission impairment (Ademiluyi et al. 2016a). Furthermore, scopolamine (i.p.) administration at 1 mg/kg has been observed to reduce ecto 5-nucleotidase activity accompanied by a memory impairment (Marisco et al. 2013). It is therefore hypothesized that the inhibition of the ecto 5-nucleotidase activity observed in the alkaloid extracts in the present study can be compared to that observed in scopolamine administered by Marisco et al. (2013), suggesting that the alkaloid extracts mediated a similar pathway similar to that of scopolamine. This is even consistent with the fact that *D. stramonium* contains a tangible scopolamine content. Meanwhile, the ability of *C. papaya* (male and female) to alter the purinergic system activity can be associated with its metal chelating abilities as observed in the present study. This finding is consistent with that observed by Da Silva et al. 2006, stating that ecto-5-nucleotidase/CD73 and ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase) enzymes depend on divalent metal ions to achieve maximal catalytic activities. Na^+/K^+-ATPase is the enzyme implicated in the maintenance of electrochemical gradients across cell membranes. Aberration of this equilibrium was observed by de Lores Arnaiz and Ordieres (2014) to cause depolarization of nerve endings, accompanied by the influx of calcium ions into the cells and thereby, the exit of neurotransmitters with resultant neuronal swelling. The present study observed a significant inhibition of the enzyme, Na^+/K^+-ATPase, and results were of the pattern *D. stramonium* > *C. sativa* > *N. tabacum* > *C. papaya* (male) > *C. papaya* (female) in descending order of Na^+/K^+-ATPase activity inhibition.

Furthermore, the present study observed that incubation of rat brain homogenate with the alkaloid extracts of *Datura stramonium* caused the highest significant ($p < 0.05$) elevation of TBARS production ex vivo followed by *Cannabis sativa*, then *Nicotiana tabacum*, *Carica papaya* male, and *Carica papaya* female respectively. TBARS production has been implicated as a major diagnostic index of lipid peroxidation in neurodegeneration. Lipid peroxidation is the free radical-arbitrated oxidation of polyunsaturated fatty acids that involves chain reactions that ultimately result in deleterious effects on essential biological macromolecules. Studies have it that this oxidative reaction results in lipid hydroperoxide formation which is characterized by further degradation into several aldehydic compounds such as hydroxyalkenals (Panigrahi et al. 2018).

Conclusion

The present study was able to show that alkaloid extracts of *Cannabis sativa*, *Nicotiana tabacum Datura stramonium*, and *Carica papaya* (male and female) were able to modulate the enzyme activities of the monoaminergic, cholinergic, and purinergic systems, and the oxidative stress levels of the rat brain ex vivo in a concentration-dependent manner. The inhibitory effects and elevated lipid peroxidation observed in the present studies by the alkaloid extracts of *Datura stramonium*, *Cannabis sativa*, *Nicotiana tabacum*, and *Carica papaya* (male and female) might be the underline mechanism by which they elicit psychoactivity as observed in folklore and the neurotoxicity observed in abusers during clinical observations. However, in vivo and clinical studies are recommended to ascertain the neurological and toxicological effects of the alkaloid extracts at the cellular and molecular levels.

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Declarations

Statement of animal welfare 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.

Conflict of interest The authors declare no competing interests.

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