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Robust congregation influences of AB-PINACA and MDMB-4en-PINACA inhalation on neurobehavioral and cardiac disorders in mice

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Abstract

Background A novel category of unusual cannabinoid substances was created to serve as marijuana alternatives due to its widespread availability, low price, pleasurable effects, and difficulty to be detected in regular urine testing for drugs, although more potential for abuse, toxicity, and behavioral alterations can result. It is more hazardous to multiple organ systems and has higher CB1 and CB2 receptor affinities than natural cannabinoids. So, the abuse potential, toxicity, and cardiac and nervous systems health hazards of two popular street herbs (AB-PINACA and MDMB-4en-PINACA) have been evaluated in mice.

Methods Thirty male mice were separated into three equally sized groups indiscriminately: the control group: received no treatments, the AB-PINACA-treated group, and the MDMB-4en-PINACA-treated group. Treated groups were exposed to the two herbs for two consecutive days via inhalation to simulate natural human exposure. Cannabinoid tetrad tests and anxiety-like behavior were performed. Serum samples were obtained for cardiac enzymes measurement. Heart and brain tissue samples were harvested for the determination of oxidative stress markers, brain neurotransmitters, and histopathological findings.

Results Nociception and hypothermia were significantly influenced by both treatments. The locomotor activity decreased significantly with AB-PINACA inhalation, while the cataleptic effect increased significantly with MDMB-4en-PINACA inhalation. In addition, both treatments induced anxiety-like behavior. Both treatments induced alterations in brain neurotransmitter levels (glutamate, dopamine, and serotonin) and cardiac enzyme levels (CK-MB, troponin I). Histological changes showed neurodegenerative, necrotic, and infracted heart myocytes and degenerated muscle fibers, particularly with MDMB-4en-PINACA inhalation.

Conclusions Acute inhalation of street herbs containing AB-PINACA and MDMB-4en-PINACA induced neurobehavioral and cardiac disturbances, which were evident by changes in behavior, brain neurotransmitters, and heart enzymes, in addition to the degenerative histopathological changes in the brain and heart.

Keywords Synthetic cannabinoids, MDMB-4en-PINACA, AB-PINACA, Behavior, Brain neurotransmitters, Cardiac enzymes, Histopathological changes

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1 Background

A novel category of unusual cannabinoid substances was created to serve as marijuana alternatives due to its widespread availability, low price, pleasurable effects, and difficulty to be detected in regular urine testing for drugs, SC abuses have dramatically increased over time [1, 2]. As with 9-tetrahydrocannabinol (9-THC) present in marijuana, these synthetic cannabinoids work directly at cannabinoid CB1 and CB2 receptors. However, they feature distinct chemical compositions independent of 9-THC, and different metabolic processes, and are often associated with higher levels of toxicity [3].

Synthetic cannabinoids (SCs) initially emerged as recreational substances in Europe with the commercial name "Spice" in 2006, and then from 2009, their consumption has expanded in the US under the commercial name "K2". SCs have recently gained popularity among teens and young adults in the USA, Europe, and Australia as recreational substances [2, 4, 5].

There are four different generations of SCs. In terms of synthesis, the first generation was the pioneering; those full agonists for CB1 and CB2 had greater affinities over THC and produced more profound dopamine-stimulating effects, such as JWH-018. The JWH series' other members, like the JWH-210, are included in the second generation that exhibits a much greater affinity for CB receptors. The third generation, including ABCHMINACA and MDMB-CHMICA, has the greatest affinity for the CB1 receptor. The 4F-MDMB-BINACA and 4F-ABINACA belong to the fourth-generation SCs, where there are neither published pharmacokinetic data for them nor available clinical reports regarding their toxicological outcomes [6–10].

The seriousness and extent of SCs' psychological impacts, which remain unclear and poorly documented in studies, have raised concerns about the fatalities and dangerous consequences associated with them all over the globe. The SCs are considerably more toxic compared to THC and produce more long-lasting consequences as a result of their greater binding to CB1 receptors [10].

MDMB-4en-PINACA and MMB-4en-PICA are two new future-generation SCs with alkene tails that were both discovered in Europe and the US during 2018 and 2019 [11]. In April 2019, Erol Ozturk and Yeter [12] reported finding MDMB-4en-PINACA in a Turkish herbal preparation.

More recent SCs referred to as AB-PINACA have just been listed as a restricted chemical in the US [13]. As reported by Thornton et al. [14], a 10-month-old young child who accidentally inhaled the SCs AB-PINACA developed neurological depression and required artificial respiration. A 10-month-old baby girl who was seen chewing on a K2 cigarette but had no history of illness appeared at the Emergency within 30 min of getting found by her mother. The findings of the medical exam were non-specific and normal. However, the infant ceased reacting to verbal and tactile cues after 90 min and suffered respiratory depression that necessitated hospitalization, while preliminary testing results from the study were unremarkable.

AB-PINACA presently has a small number of in vitro and in vivo researches [15]. Wiley et al. [16] and Kevin et al. [17] assessed the in vivo effects of novel SCs such as THC, AB-PINACA, and AB-FUBINACA in mice and rats and reported that these compounds showed a higher affinity to CB1 receptors, suppressed locomotor activity, catalepsy, antinociception, hypothermia, and increased anxiety-like behaviors at high and low doses.

Moreover, MDMB-4en-PINACA has few in vivo studies for pharmacological or toxicological effects and abuse potential [18, 19]. Thus, the acute neurotoxicity of MDMB-4en-PINACA and AB-PINACA has been investigated in this study via employing some rodent behavioral tests, in addition to assessing the changes in the histopathology and biochemical parameters for evaluation of neurobehavioral and cardiac disorders in mice.

2 Methods

2.1 Animals

Mice were purchased and kept in a well-ventilated environment, in plastic polypropylene cages, and fed a commercial balanced diet and clean freshwater continuous adequate supply. The mice were given 10 days for adaptation. A digital hygrothermometer was used to measure the temperature and relative humidity (RH), with the temperature ranging between 19–22.5 °C and RH between 40–55%, and the lighting source was kept on a 12-h alternating light–dark pattern. Ethics guidelines were provided for this experiment by the Institutional Animal Care and Use Committee.

2.2 Herbal substances

Before banning legalization, two herbal SCs samples were given to the faculty of Medicine toxicology lab for isolation and extraction.

2.2.1 Herbal substance extraction

By mixing 10 mg of the herbal mixture with 1 mL of methanol and sonicating it for 10 min, MDMB-4en-PINACA and AB-PINACA were obtained [20]. Methanol solvent was of analytical grade and purchased from Sigma-Aldrich, Egypt.

2.2.2 Volatilization of herbal substances

A non-published questionnaire was held for attendees to our lab about abusing synthetic herbal substances by

inhalation to determine the average amount of herbs used daily by each person. The average amount was 2 g per cigarette for each 100 kg person. A matrix of Trans-Blot Transfer Medium Pure Nitrocellulose Membrane was used to make cannabinoids volatile in the chamber. MDMB-4en-PINACA and AB-PINACA were first dissolved in 100% concentrated ethanol, and then the dissolvent was put into nitrocellulose paper (8.0 8.0 cm squares) all night in the fuming hood. Cannabinoids that were impregnated in the nitrocellulose paper were left after the ethanol evaporated. MDMB-4en-PINACA- and AB-PINACA-inhaled cannabis dosages were mg per 30 L of the chamber's air [21].

2.3 Inhalation apparatus

The inhalation apparatus was built and designed, and the guidelines of the processes were performed to simulate the real way in abusers according to Marshell et al. [21].

2.4 Experimental design

A total of thirty male Swiss albino mice weighing approximately 25–35 g are randomly separated into three groups (each group contains 10 mice): control group: not exposed to any treatments; AB-PINACA group: mice inhaled substance once daily for two successive days; and MDMB-4en-PINACA group: mice inhaled substance once daily for two days. Mice exposed to inhalation of AB-PINACA and MDMB-4en-PINACA once daily for two successive days with a dose of 0.02mg/ gm in adjusted space as 1mg of drug per 30 L of air in the inhalation apparatus. Mice prepared for the rectal probe insertion two days before the start of the experiment by recording their baseline temperature.

2.5 Behavioral tests

Cannabinoid tetrad tests and anxiety-like behavior were carried out 30 min after exposure to the AB-PINACA and MDMB-4en-PINACA via inhalation.

2.5.1 Cannabinoid tetrad tests

2.5.1.1 Locomotion Locomotion was measured by open field maze (OFT). The maze was cleaned using alcohol after each mouse was removed, and a video recording of the mice's movement across the maze took place by placing every mouse in the corner of the maze and leaving 5 min for exploration [22, 23].

2.5.1.2 Body temperature To estimate the incidence of hypothermia, the temperature of the body was measured by digital thermometer insertion into the rectum. The basal rectal temperature was monitored 5 min before and 50 min after inhalation exposure [24, 25].

2.5.1.3 Catalepsy test Place the mouse in a catalepsy cage. The mouse's front paws were positioned slowly on the horizontal rod, and its hind legs were positioned carefully on the floor of the cage to determine if it was in catalepsy. Until the mouse slips down the bar or the cutoff time (300 s) has elapsed, keep time with the aid of a stopwatch. The catalepsy cage was scrubbed using ethanol at a concentration of 20% following the removal of each mouse [26].

2.5.1.4 Tail immersion test The tail distal extremity was submerged in a 56 °C water bath to measure antinociception. A cutoff time of 10 to 12 s was used to measure the delay for the tail withdrawal from the water [25, 27].

2.5.2 Elevated plus maze to assess anxiety in mice

Rodent anxiety is assessed by the elevated plus maze (EPM) [28, 29]**.** The maze is composed of four arms (two of which are open while the other two are enclosed by walls). Each mouse was positioned in the center of the apparatus and given 5 min to explore it, and after each mouse evaluation, the maze arms were wiped down with 70% ethyl alcohol, and then recorded videos were analyzed according to [29].

2.6 Blood and tissue sampling

At the end of the behavioral tests, blood samples were collected to obtain serum for measurement of cardiac enzyme levels, and mice were sacrificed humanely by decapitation under light anesthesia by intraperitoneal injection of 50 mg/kg Ketamine. Then, the heart and brain were extracted and then were cut into two portions. One portion was preserved in 10% buffered formalin fixative for 48 h for histopathological examination, and the other portion was kept at -80°C to be homogenized for estimations of the biochemical biomarkers (oxidative stress and brain neurotransmitters).

2.6.1 Biochemical assay

2.6.1.1 Oxidative stress indicators measurement Following Uchiyama and Mihara [30], the concentration of malonaldehyde (MDA) in the heart and brain was determined at 520–535 nm in n-butanol colorimetrically. In accordance with Sedlak and Lindsay [31], the concentration of glutathione (GSH) was also determined colorimetrically by using Ellman's reagent. Paoletti et al. [32] method was used to assess the concentration of superoxide dismutase (SOD) in the brain and cardiac tissues.

2.6.1.2 Brain neurotransmitters measurement Glutamate was colorimetric ally measured using Enzy-

Chrom™ Glutamate Assay Kit. Serotonin and dopamine were measured biochemically using ELISA kits.

2.6.1.3 Measurement of heart enzymes In accordance with Young [33] and Collinson et al. [34], protein kinase C (CK-MB) and troponin I cardiac enzymes were measured using ELISA kits.

2.6.2 Histopathological examination

The brain and heart of the mice were removed after the study, and they were immediately placed for 48 h in a 10% buffered formalin fixative. According to Suvarna et al. [35], the collected samples of brain tissue have been subjected to the usual paraffin embedding process and 5 m thick serial coronal slices of the brain as well as longitudinal slices of the heart were stained.

2.6.3 Brain

Serial coronal slices of the brain were stained by three stains: first for histopathological lesions general examination and scoring by hematoxylin and eosin stain (H&E), second for endocrine secretion and nerve fibers identification by silver impregnation stain, and third for cytoplasmic total protein visualization by bromophenol blue stain.

2.6.4 Heart

Longitudinal slices of the heart were stained with three stains: H&E stain for general assessment, as well as scoring of the histopathological lesions, mucin identification by periodic acid schiff stain (PAS), and cytoplasmic total protein visualization by bromophenol blue stain.

With a LEICA (DFC290 HD) digital camera, the stained portions have been captured on video. According to Gibson-Corley et al. [36], pathological lesions were scored under a light microscope as follows: − = zero pathological abnormalities and looked normal; −/+ = abnormalities < 25%; + = abnormalities in 26–50%; ++ = abnormalities in 51–75%; and +++ = abnormalities > 75%).

2.7 Statistical analysis

By using SPSS version 22 statistical software, the recorded data were tested for normality, followed by one-way ANOVA, and post hoc testing (Tukey and Dunnett test) for statistical analysis of biochemistry parameters, and behavioral analysis by nonparametric Kruskal–Wallis test. Data are shown as (mean ± standard error (SE) of the mean for tables or mean ± standard deviation (SD) of the mean for figures) and the significant difference was considered when $P < 0.05$.

3 Results

3.1 Behavioral tests

From Fig. 1, it was clear that exposure to AB-PINACA and MDMB-4en-PINACA through inhalation produced a classic tetrad cannabinoid effect in mice. The obtained results showed that AB-PINACA inhalation significantly diminished locomotor activity by a reduction in the number of square crossing ($P = 0.02$) in the OFT in mice in relation to the control group, while MDMB-4en-PINACA inhalation showed no effect on the locomotion in OFT compared to the control group, but a significant ($P = 0.02$) elevation in locomotion appeared when compared to AB-PINACA group (Fig. 1A).

In addition, a significant ($P = 0.02$) hypothermia was detected in mice after exposure to inhalation of both AB-PINACA and MDMB-4en-PINACA than in the control group (Fig. 1B). Moreover, it was observed that MDMB-4en-PINACA increased total latency in the catalepsy cage at a significant difference ($P = 0.05$) in comparison with control and AB-PINACA groups. On the other side, AB-PINACA also visually increased latency to move in relation to the control group but no statistical difference was found (Fig. 1C). The inhalation of both AB-PINACA and MDMB-4en-PINACA (Fig. 1D) also revealed a significant ($P = 0.02$) delay in the latency for tail removal in the tail immersion test.

From Fig. 2, the obtained data revealed that inhalation of AB-PINACA and MDMB-4en-PINACA induced anxiety-like behavior in EPM which was noticed by the reduction of time spent in EPM open and closed arms by mice which made a significant difference ($P = 0.03$, $P = 0.02$), respectively, with AB-PINACA compared to control mice (Fig. 2A). In addition, inhalation of MDMB-4en-PINACA decreased the open and closed arm duration, but a significant difference ($P = 0.02$) between MDMB-4en-PINACA and control groups only in time spent in closed arms. In both groups of herbal cannabinoid inhalation, the number of open arms entrances reduced dramatically ($P = 0.05$) as compared to control mice, and also the number of closed arms entrances reduced non-significantly in the inhaled groups as compared to control mice (Fig. 2B).

3.2 Biochemical parameters

Table 1 reveals that GSH, SOD, and MDA levels in the brain did not differ between all groups with the absence of statistical difference between all groups. In addition, no statistical difference was also present between AB-PINACA and MDMB-4en-PINACA and control groups in the oxidative stress parameters (GSH, SOD, MDA) in the heart.

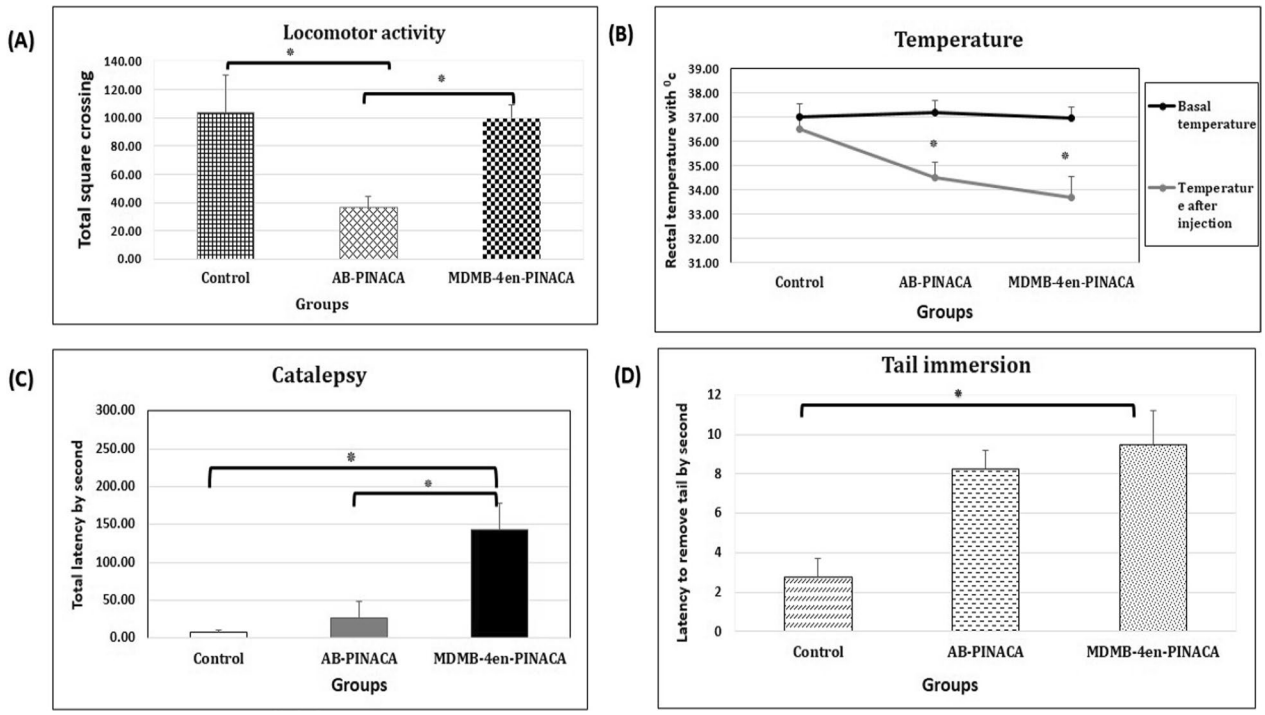

Fig. 1 Cannabinoid tetrad tests: (A) locomotion, (B) rectal temperature, (C) catalepsy and (D) tail immersion. Data expressed as mean ± SD. When a P-value is less than 0.05, a star on the graph denotes statistical significance. Locomotion ($n = 4$; $df = 2$; chi-square = 7.446); rectal temperature ($n = 4$; $df = 2$; chi-square = (0.746, 8.405); catalepsy ($n = 3$; $df = 2$; chi-square = 6.006); tail immersion ($n = 4$; $df = 2$; chi-square = 8.200)

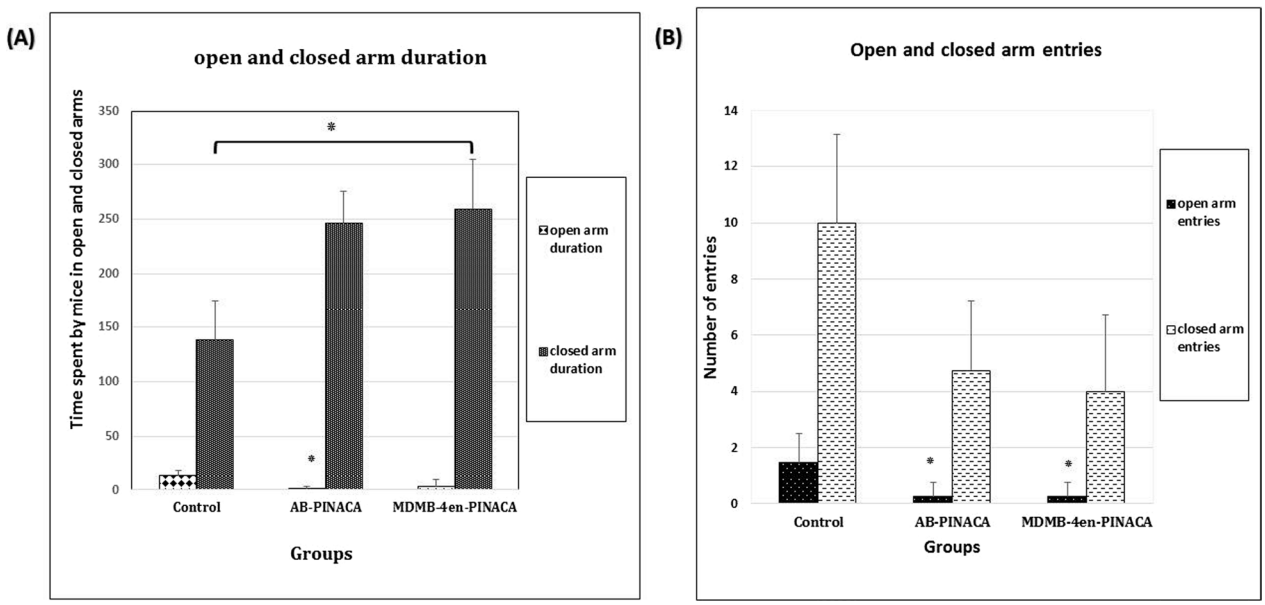

Fig. 2 Effect of PINACA inhalation on mice anxiety which includes: the mice's time in open and closed arms is shown in **A**, and the mice's open and closed arm entries into the elevated plus maze are shown in **B**. Data expressed as mean ± SD. When a *P* value is less than 0.05, a star on the graph denotes statistical significance. Open and closed arm duration ($n = 4$; $df = 2$; chi-square = 6.567, 8.000 in order); open and closed arm entries ($n = 4$; $df = 2$; chi-square = (6.036, 5.869, respectively)

Table 1 Acute effect of AB-PINACA and MDMB-4en-PINACA inhalation on mice brain and heart oxidative stress parameters

Groups	Parameters			
	MDA (ng/ml)	GSH (ng/ml)	SOD (ng/ml)	Significance
Brain				
Control	0.14 ± 0.01	0.02 ± 0.00	27.95 ± 1.82	NS
AB-PINACA	0.17 ± 0.01	0.02 ± 0.00	34.20 ± 1.79	NS
MDMB-4en-PINACA	0.15 ± 0.01	0.01 ± 0.00	21.75 ± 1.82	NS
Heart				
Control	0.23 ± 0.02	0.01 ± 0.00	52.85 ± 1.76	NS
AB-PINACA	0.23 ± 0.03	0.01 ± 0.00	59.25 ± 1.93	NS
MDMB-4en-PINACA	0.21 ± 0.02	0.01 ± 0.00	46.60 ± 1.79	NS

Results are expressed as means ± SE. *NS* non-significant, *MDA* malonaldehyde, *GSH* glutathione *SOD* superoxide dismutase

Table 2 Acute effect of AB-PINACA and MDMB-4en-PINACA inhalation on brain neurotransmitters levels in mice

Groups	Parameters		
	Glutamate (ng/ml)	Serotonin (ng/ml)	Dopamine (ng/ml)
Control	0.98 ± 0.03	10.63 ± 0.38	1.56 ± 0.05
AB-PINACA	2.34 ± 0.05*	6.75 ± 0.27*	3.75 ± 0.07*
MDMB-4en-PINACA	3.04 ± 0.04*	4.10 ± 0.38*	4.86 ± 0.06*

Results are expressed as means ± SE

* Superscripts in the same column indicate significance with the control group at $P = 0.00$

The obtained data in Table 2 revealed that inhalation of AB-PINACA and MDMB-4en-PINACA increased the brain level of glutamate significantly ($P = 0.00$) than the control group. Moreover, the dopamine level increased significantly ($P = 0.00$) in AB-PINACA and MDMB-4en-PINACA groups in relation to a control group. However, serotonin levels decreased significantly ($P = 0.00$) in AB-PINACA and MDMB-4en-PINACA groups when compared with the control group.

From Table 3 it was observed that AB-PINACA and MDMB-4en-PINACA inhalation caused an elevation in cardiac enzyme levels in serum. The CK-MB level increased significantly at ($P = 0.00$) in both AB-PINACA and MDMB-4en-PINACA groups than the control group. Also, Troponin I level increased in both AB-PINACA and MDMB-4en-PINACA groups significantly at ($P = 0.00$) in relation to a control group.

3.3 Histopathological findings

In the current investigation, Table 4 and Figs. 3, 4, and 5 demonstrate the differences in the effects of both inhaling AB-PINACA and MDMB-4en-PINACA chemicals on the brain and heart tissue of mice.

Table 3 Acute impact of AB-PINACA and MDMB-4en-PINACA inhalation on serum cardiac enzymes levels

Parameters	CK-MB (U/L)	TROPONIN I (ng/mL)
Groups		
Control	12.70 ± 0.40	0.15 ± 0.02
AB-PINACA	24.38 ± 0.95*	0.59 ± 0.06*
MDMB-4en-PINACA	30.01 ± 0.89*	1.09 ± 0.06*

Results are expressed as means ± SE

* Superscripts in the same column indicate significance with the control group at $P = 0.00$

CK-MB creatine protein-MB

3.4 The brain

In Figure 3, H&E stained cerebrum sections showed fine fibrous meningeal layers with normal capillaries in the control mice (Fig. 3A, A1), a normal arrangement of the cerebral cortex sex layers, as well as normal neurons with vesicular nuclei and basophilic cytoplasm, neuroglia cells, and capillaries. While in the AB-PINACA group, the meninges were destructed with vascular congestion,

Table 4 Semi-quantitative histopathological scoring of all alterations appeared microscopically in cardiac and brain tissues of herbal used mice compared with control group by staining sections with H&E X100

Organs and lesions	Control	AB-PINACA	MDMB-4en-PINACA
Brain			
Vascular congestion	–	++	+++
Neuronal degeneration	–	++	+++
Chromatolysis	–	++	+
Spongiosis	–	+	++
Gliosis	–	–	+
Heart			
Congestion	–	+++	+++
Edema	–	++	++/+++
Myocytic degeneration	–	++	+++
Vacuolation	–	++	++
Infarction	–	+	++
Thrombosis	–	+	++

Absence of lesions (–), minimal degree (–/+), mild degree (+), moderate degree (++) and sever degree (+++)

the appearance of mild spongiosis, most of the cerebral neurons and neuroglia showed degenerative and necrotic changes while few appeared normal (Fig. 3B, B1); also, most of the cerebral blood vessels degenerated and congested. Furthermore, in the MDMB-4en-PINACA group, all the former lesions have appeared but with extreme severity, in addition to the appearance of gliosis and focal interstitial hemorrhage with inflammatory cell infiltration (Fig. 3C, 3C1)

Hippocampus H&E stained sections: in the control mice, the stratum pyramidal showed normal pyramidal cells with central vesicular nuclei and clear nucleoli (Fig. 3A2). However, some degenerated pyramidal cells with pyknotic nuclei appeared in the AB-PINACA group (Fig. 3B2), while the majority of necrotic changes appeared in MDMB-4en-PINACA group pyramidal cells (Fig. 3C2).

Cerebellum H&E stained sections: the Purkinje cells appeared fusiform with normal nuclei and nerve fibers in control mice (Fig. 3A3). In the AB-PINACA group, some degenerated Purkinje cells without nuclei present, and some appeared normal (Fig. 3B3). In the MDMB-4en-PINACA group (Fig. 3C3); major necrotic changes appeared in Purkinje cells with pyknotic nuclei or without nuclei.

In Fig. 4 sections stained with silver stain: representative brain tissue sections revealed that, in the control group, the nerve fibers appeared black in the form of parallel bundles. Fibers appeared long, thick, and highly branched with numerous synapses (Fig. 4A, A1). But, AB-PINACA group shows thin bundles of short, thin, less branched nerve fibers, and short single ones with few synapses (Fig. 4B, B1). The MDMB-4en-PINACA group showed few thin bundles of thin nerve fibers, and most fibers appeared single, thin, short, and non-branched run in random distribution (Fig. 4C, C1).

Sections stained with Bromophenol blue: representative brain tissue sections showed that all nerve cells revealed a strong positive reaction of blue color in control mice (Fig. 4A2). However, some nerve cells exhibited moderate reaction and others appeared with mild reaction in the AB-PINACA group (Fig. 4B2), as well most of the nerve cells showed minimal reaction and few appeared with mild reaction in the MDMB-4en-PINACA group (Fig. 4C2).

3.5 The heart

H&E-stained sections: in the control group, the cardiomyocytes showed normal arrangement in the form of short, branching, and anastomosing cylinders with acidophilic cytoplasm and oval central vesicular nuclei (Fig. 5A, A1). In the AB-PINACA group, the myocytes showed moderate necrotic changes with pyknotic nuclei and intracellular vacuolations. The vascular lining was degenerated, and the vessels either showed severe congestion or closed with a thrombus. There were some infarcted myocytes with faint cytoplasm, inflammatory cell infiltrations, and interstitial edema (Fig. 5B, B1). In the MDMB-4en-PINACA group, multifocal inflammatory cell infiltrations appeared, and all aforementioned pathological alterations in the AB-PINACA group were observed, but to a severe degree (Fig. 5C, C1).

PAS-stained sections: the cardiomyocytes exhibited strong pink color indicating normal mucopolysaccharides content in control mice (Fig. 5A2). However, in the AB-PINACA-treated mice, the degenerated muscle fibers showed a moderate reaction (Fig. 5B2), and the reaction was mild in the MDMB-4en-PINACA-treated sections (Fig. 5C2).

Bromophenol blue-stained sections: in the control mice, the cardiomyocytes revealed a strong blue color indicating normal protein content (Fig. 5A3). In the AB-PINACA-treated mice, the degenerated muscle fibers exhibited moderate reaction (Fig. 5B3), while the reaction appeared mild in the MDMB-4en-PINACA-treated mice (Fig. 5C3).

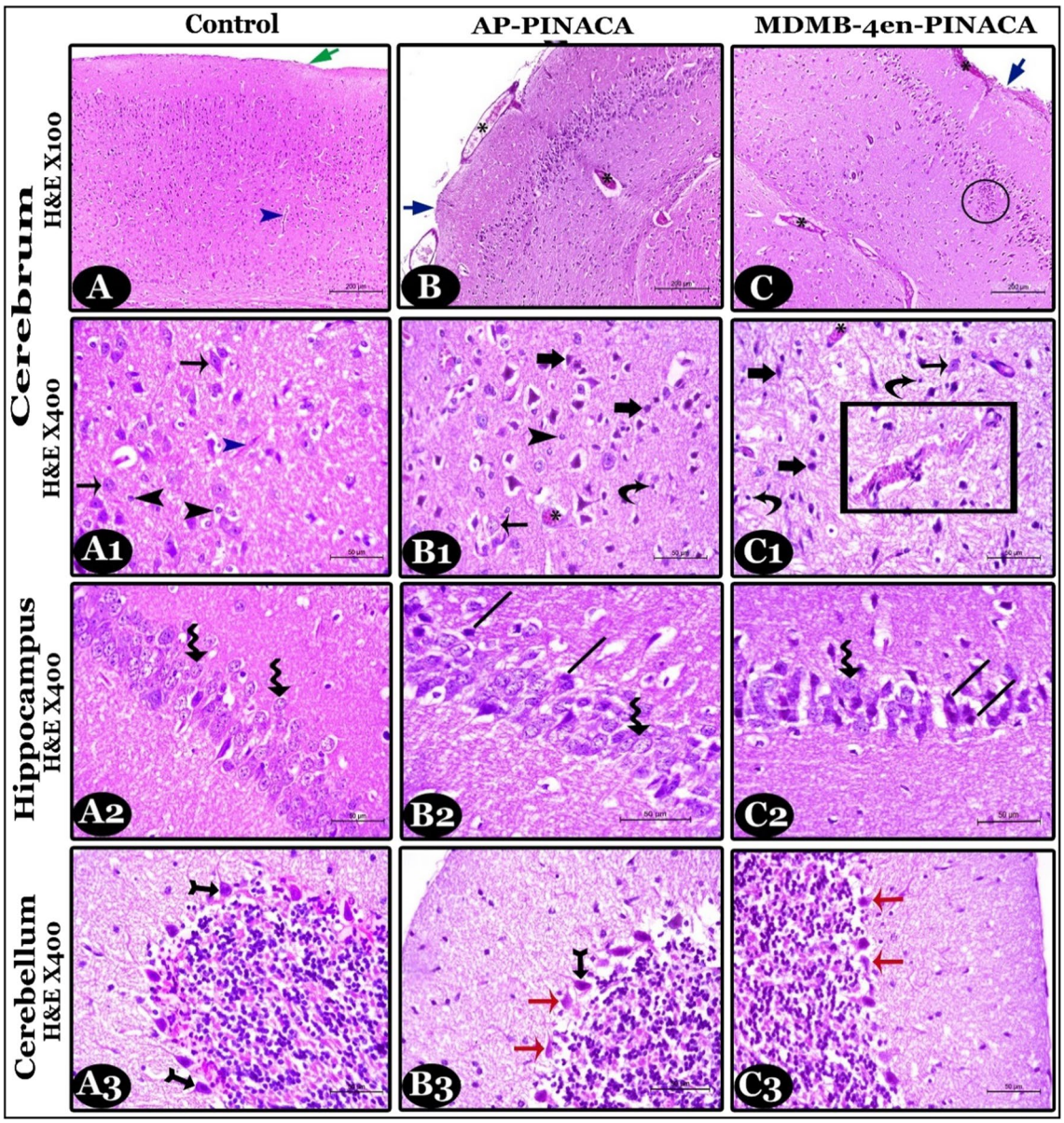

Fig. 3 Coronal mice brain tissue sections stained with H&E: Cerebrum of Control group (**A**, $\mathbf{A_1}$), AB-PINACA group (**B**, $\mathbf{B_1}$), and MDMB-4en-PINACA group (**C**, $\mathbf{C_1}$), in addition, Hippocampus (stratum pyramidal), and Cerebellum of Control group ($\mathbf{A_2}$, $\mathbf{A_3}$), AB-PINACA group ($\mathbf{B_2}$, $\mathbf{B_3}$), and MDMB-4en-PINACA group ($\mathbf{C_2}$, $\mathbf{C_3}$), showed normal meninges (green arrow), normal capillaries (blue arrowheads), normal cerebral neurons (thin black arrows), normal neuroglia cells (black arrowheads), degenerated meninges (blue arrows), degenerated cerebral neurons (thick arrows), pyknotic glia cells (curved arrows), gliosis (circle), normal pyramidal neurons (zigzagged arrows), degenerated pyramidal neurons (straight lines), normal Purkinje cells(tailed arrows), degenerated Purkinje cells (red arrows), degenerated and congested capillaries (*), hemorrhage with inflammatory cells infiltration (square). All photomicrographs X400 except A, B, and C were X100

4 Discussion

The inhalation of AB-PINACA and MDMB-4en-PINACA was found to induce behavioral changes, biochemical alteration in levels of brain neurotransmitters and cardiac enzymes, and histopathological changes [19, 37, 38].

The neurobehavioral disturbances were demonstrated by external alterations in the mice's behavior that were represented in typical cannabinoid tetrad tests and anxiety-like behavior results and reflected internally by the

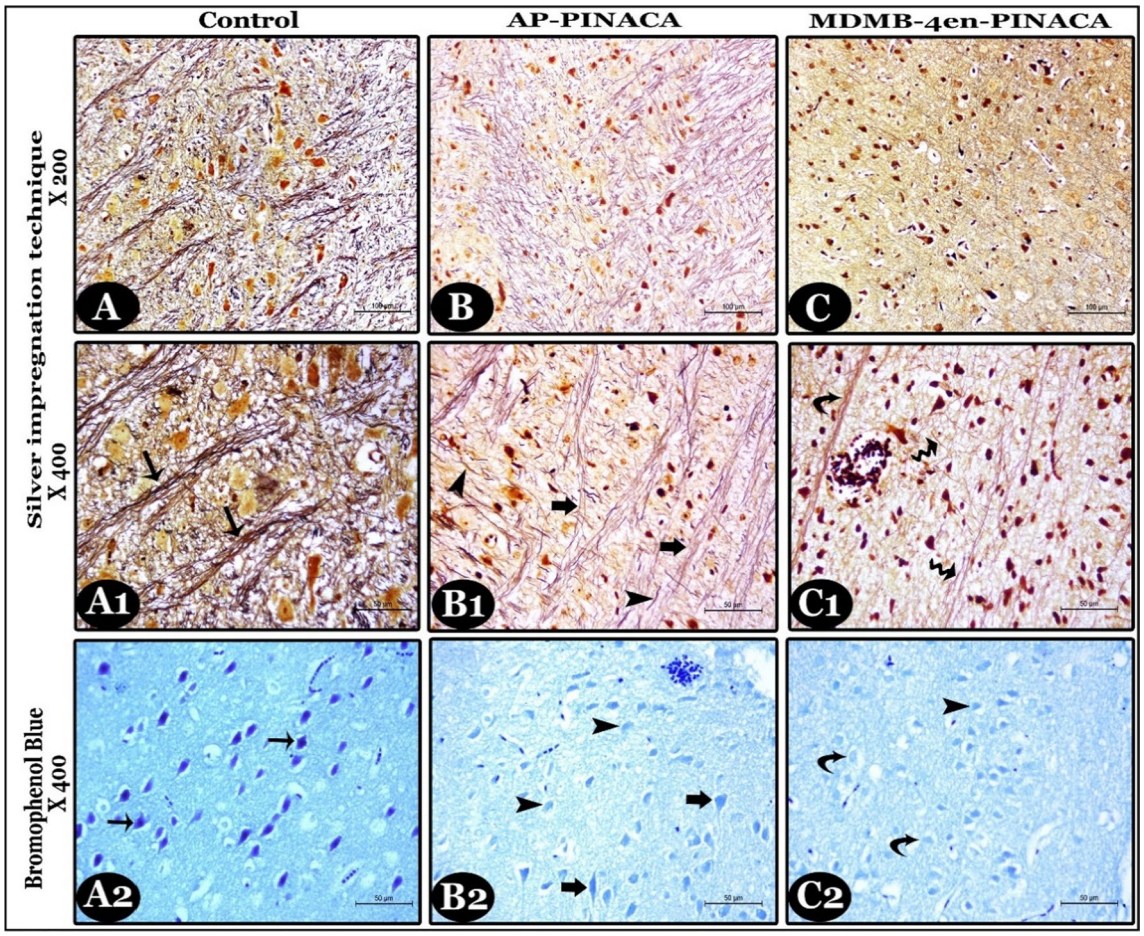

Fig. 4 Representative brain tissue sections photomicrographs from mice of all studied groups; sections stained with silver impregnation technique in the 1st raw (X200) and 2nd raw (X400); thick bundles of long, thick parallel, highly branched nerve fibers of black color (long arrows) appeared in the control group (**A**, $\mathbf{A_1}$). Thin bundles of short, thin, and less branched nerve fibers (thick short arrows) and short single ones (arrowheads) in the AB-PINACA group (**B**, $\mathbf{B_1}$). One bundle of thin nerve fibers (curved arrow) and most fibers appeared single, thin, short, and non-branched run in random distribution (zigzagged arrows) in the MDMB-4en-PINACA group (**C**, $\mathbf{C_1}$). Sections stained with Bromophenol Blue stain in the 3rd raw (X400); Control group ($\mathbf{A_2}$) showed all neurons with a significant positive reaction of blue color (long arrows). AB-PINACA group ($\mathbf{B_2}$) showed moderate reaction within some degenerated neurons (thick short arrows) and mild reaction in the others (arrowheads). MDMB-4en-PINACA group ($\mathbf{C_2}$) showed a minimal reaction in most degenerated neurons (curved arrows) and a few with mild reaction (arrowheads)

disturbances in the brain neurotransmitter levels and brain neurodegenerative changes.

The obtained data showed that inhalation of AB-PINACA and MDMB-4en-PINACA induced significant hypothermia, and greater latency in mice's tail immersion and catalepsy tests, while their effect on the locomotor activity differs, whereas MDMB-4en-PINACA showed no effect on the locomotion and AB-PINACA induced a significant hypolocomotion. Several studies indicated similar results where Banister et al. [39] noted that AB-PINACA dose-dependently induced hypothermia and bradycardia. Also, Lefever et al. [24] observed that exposure to aerosols had biphasic effects on locomotion activity, showing elevations at minimal levels and reductions at excessive levels, which resulted in hypothermic consequences. In addition, Ali et al. [19] and Long et al. [40] reported that non-psychotomimetic constituent cannabidiol (CBD), MDMB-4en-PINACA acute administration in mice did not affect any locomotor activity measure in the open field test. Moreover, Wiley et al. [16] and Javadi-Paydar et al. [41] found that inhalation of Δ9-tetrahydrocannabinol (THC), AB-PINACA, and cannabidiol (CBD) produced hypothermia, catalepsy, anti-nociception, and suppressed locomotor activity in mice and rats.

Noticing catalepsy results, Naumenko et al. [42] found that In contrast to catalepsy-resistant AKR/J mice; all mice susceptible to catalepsy had substantially

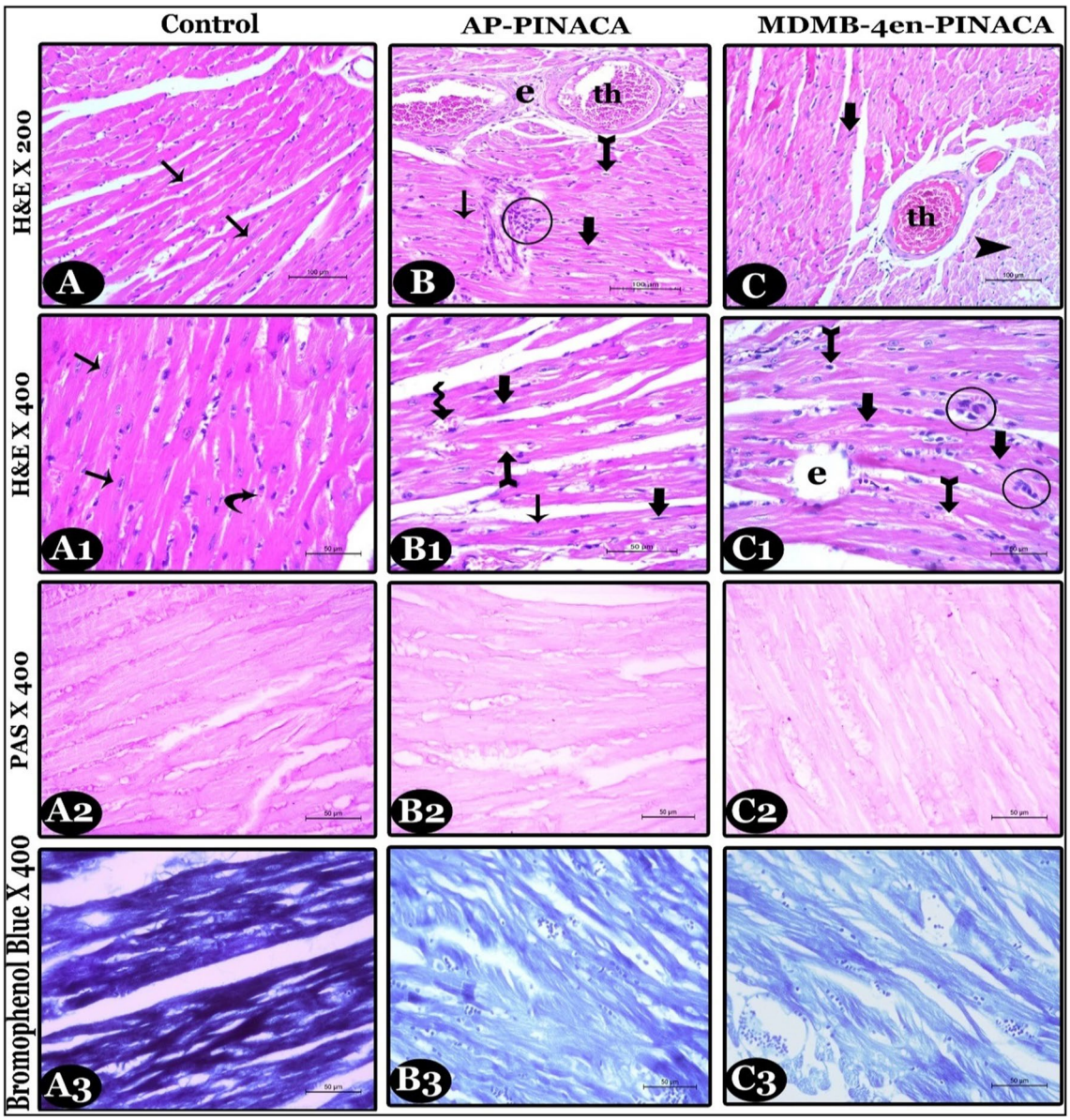

Fig. 5 Sections of heart from mice of all studied groups. Sections stained with H&E appeared in the 1st raw (X200); Control group (**A**), AB-PINACA group (**B**), and MDMB-4en-PINACA group (**C**)and 2nd raw (X400); Control group ($\mathbf{A_1}$), AB-PINACA group ($\mathbf{B_1}$), and MDMB-4en-PINACA group ($\mathbf{C_1}$): stained sections appeared as following, normal cardiac muscle cells with oval vesicular nuclei (thin long arrows), normal capillaries (curved arrows), necrotic myocytes with pyknotic nuclei (thick short arrows), infarcted myocytes (arrowheads), intracellular vacuolations (tailed arrows), edema (e), inflammatory cell infiltrations (circle), degenerated and congested blood vessels (zigzagged arrows) and thrombus (th). Sections stained with PAS stain in the 3rd raw (X400); the control group ($\mathbf{A_2}$) showed cardiomyocytes with a significant positive reaction of pink color. AB-PINACA group ($\mathbf{B_2}$) showed moderate reaction within the degenerated myocytes. MDMB-4en-PINACA group ($\mathbf{C_2}$) showed mild reaction within the degenerated myocytes. Sections stained with Bromophenol Blue stain in the 4th raw (X400); cardiomyocytes appeared with a significant blue color positive reaction in the control group ($\mathbf{A_3}$), a blue color moderate reaction in AB-PINACA group ($\mathbf{B_3}$) and a blue color mild reaction in MDMB-4en-PINACA group ($\mathbf{C_3}$)

reduced 5-HT (2A) receptor functioning, as well as decreased 5-HT (2A) receptor gene transcription in the frontal brain, which run parallel with our results as serotonin level in brain elevated as catalepsy reduced and decreased as catalepsy increased. The declined serotonin levels run in line with De Gregorio et al. [43] observed that THC exposure resulted in decreased serotonin activity which is associated with increased anxiety-related behavior and depression.

The results of our study demonstrated that the newly developed SCBs AB-PINACA elevated anxiety, as reported by Kevin et al. [17] through evaluation of anxiety-like behavior. Furthermore, THC was found to have varying impacts on anxiousness in mice based on age, dosage, and sex. In mature mice, substantial doses have been shown to raise anxiousness in EPM by decreasing both the number of entries and length of stay in open arms, whereas low dosages just decreased the length of time spent in open arms but did not alter the number of entries [44].

In addition, the obtained results showed elevated glutamate levels in inhaled groups in association with elevated levels of anxiety in EPM as reported by Onaolapo et al. [45] revealing an association between the mouse behaviors in EPM and glutamate levels with monosodium glutamate oral administration. As a result of the increased glutamate level, the mice's total arm entries, particularly open arms entrances, and the duration of time in open arms dramatically reduced. Colizzi et al. [46] demonstrated that acute administration of THC increased Glutamate levels in the head left caudate.

The obtained data showed alterations in the dopamine level that were not associated with locomotion or anxiety and these results agree with Terzian et al. [47] noting that no difference was present in the exploratory activity in OFT between D1–CB1$^{-/-}$and WT mice which indicate that genetic deletion of CB1 in neurons expressing dopamine D1 receptors (D1Rs) did not affect the mice's baseline locomotion in the EPM test with the absence of statistical significant variations between D1–CB1$^{-/-}$and WT mice. Furthermore, Ossato et al. [48] noted that SCBs stimulate the release of dopamine in mice. Hence, the suppressed locomotor activity in groups with elevated dopamine levels may be attributed to dopamine (DA) neurons firing in two unique modes, tonic and phasic, each of which is hypothesized to control different aspects of behavior. Phasic DA neuron activity disturbance reduced learning of both unpleasant and rewarding conditioned behavioral responses but did not influence learning of many other DA-dependent behaviors [49].

Concerning the histopathological changes in the brain, Esawy et al. [50] described pathological changes in rat brain induced by AMB-FUBINACA as a focal area of pyknotic, severe hemorrhage, apoptosis, and inflammatory cell aggregates in the cerebral cortex and hippocampus. Moreover, Karakayali [51] mentioned that SCBs increased the incidence of ischemia and hemorrhagic strokes in the nervous system.

Synthetic cannabinoids are binding with high affinity to one of the two known end cannabinoid receptors, CB1 or CB2 receptors. In this study, the non-significant difference in oxidative stress parameters measured in the mice brain and heart was supported by Abdel-Salam et al. [52] and Kopjar et al. [53] which observed that acute administration of SCBs (fluoxetine and THC) didn't alter the brain and plasma GSH and MDA levels in rodents. Moreover, Abdel-Salam et al. [54] reported that Δ9-tetrahydrocannabinol didn't affect the heart GSH or MDA levels.

The cardiac disturbances were evident with the changes in the serum cardiac enzyme levels (CK-MB and Troponin I) and pathological abnormalities in the cardiomyocytes. Cardiac troponins I and creatine kinase isoenzyme MB (CK-MB) are among the serum indicators with diagnostic potential to identify the cardiac side effects of medications. Cardiac troponins are regarded as the most reliable indicators of heart damage as a result of their high sensitivity and specificity [55, 56].

The obtained data that run in agreement with Nnodim-Johnkennedy et al. [57] mentioned that exposure to marijuana affected cardiac enzyme levels, increasing the level of creatine kinase (CK) in the heart, and causing myocardial infarction. On the other side, Kicman and Toczek [58] discovered that certain cannabis has a cardioprotective effect in rabbits with heart disease by lowering cardiac enzyme levels (CK and troponin) and oxidative stress which may be returned to the cannabis compound, dose, disease status, and species difference. Concerning the histopathological changes, Hancox et al. [59] revealed that the acute administration of SCBs was associated with acute myocardial infarction and cardiomyopathy.

5 Conclusions

The inhalation of AB-PINACA and MDMB-4en-PINACA has severely damaging effects on health as they cause sudden decreases in body temperature, lack of feeling of pain, and depression. Moreover, they induce disturbances in cardiac enzymes and brain neurotransmitter levels which cause abrupt acute inflammatory changes in the heart and brain.

Abbreviation

SCs Synthetic cannabinoids

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Author contributions

All authors have read and approved the final manuscript. All authors (MAA, DAH, SSG, SMZS, AKA, AMM, RMH, and AGSA) equally contributed to this study. The conception or design of the work: MAA, DAH, SMZS, and AGSA shared the work conception and design. Analysis: AKA performed the analysis of behavior; MAA, DAH, and AGSA carried out the biochemical analysis, AMM and RMH carried out the histopathological analysis, and AKA and MAA

performed the work statistical analysis. Interpretation, work drafting, and revision of data: MAA, SMZS, AKA, AMM, RMH, and AGSA shared data writing and revision. Manuscript reviewing: SSG reviewed the manuscript.

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Availability of data and materials
The datasets generated and/or analyzed during the current study are available from the corresponding author if needed.

Declarations

Ethics approval and consent to participate
Ethics guidelines were provided for this experiment by the Institutional Animal Care and Use Committee at Beni-Suef University (BSU-IACUC) (permission No. 021-204).

Study design
Thirty male mice were separated to three equally sized groups indiscriminately: the control group: received no treatments, the AB-PINACA-treated group, and the MDMB-4en-PINACA-treated group. Treated groups exposed to the two herbs for two consecutive days via inhalation to simulate natural human exposure. Cannabinoid tetrad tests and anxiety-like behavior were performed. Serum samples were obtained for cardiac enzymes measurement. Heart and brain tissue samples were harvested for the determination of oxidative stress markers, brain neurotransmitters, and histopathological findings.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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