The Acute Effects of Cannabis on Cardiac Arrhythmia and Myocardial Injury Induced by Epinephrine in the Rat

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Abstract: - Cannabis, the most common illicit substance worldwide, has been associated with acute cardiovascular events such as arrhythmia including premature ventricular contractions, ventricular tachycardia, sinus arrest, and myocardial infarction. In this study, we investigated the effects of cannabis extract on electrocardiographic parameters and cardiac histology in normal rats and in an epinephrine-induced arrhythmia and myocardial damage anesthetized rat model. The possible modulation of cannabis effects by the nitric oxide synthase inhibitor *NG*-nitro-L-arginine methyl ester (L-NAME) was also examined. Male Sprague-Dawley rats were treated with a single intraperitoneal (i.p.) dose of cannabis (equivalent to Δ^9-tetrahydrocannabinol content of 20 mg/kg), prior to intravenous (i.v.) epinephrine (10 µg/kg) injection. In another group, cannabis (20 mg/kg, i.p.) was administered prior to L-NAME (40 mg/kg, i.p.) and epinephrine (10 µg/kg, i.v.). The effects of cannabis in normal rats were also investigated. The control group received saline. Results indicated that (i) the administration of a single dose of cannabis at 20 mg/kg in normal rats slowed the heart rate by 12.3%, widened the QRS complex by 110.5%, and caused a depressed ST segment, compared with the corresponding saline control; (ii); cannabis given prior to i.v. epinephrine didn't change the heart rate or QTc interval, but decreased the PR interval by 23.2%, decreased QRS duration by 30%, increased R wave amplitude by 50%, induced significant depression of the ST segment and wide QRS complex ventricular premature beats compared with the corresponding epinephrine control group; (iii) cannabis significantly increased the number and duration of epinephrine ventricular premature contractions and this showed further increase by pretreatment with L-NAME. Collectively, these results show that acute administration of cannabis in high doses caused slowing of heart rate and ST changes in normal rats, suggestive of myocardial ischemia, and increased ventricular arrhythmia induced by epinephrine. L-NAME increased ventricular arrhythmia caused by cannabis/epinephrine.

Key-Words: - cannabis; cardiac arrhythmia; epinephrine; myocardial injury; nitric oxide; nitric oxide synthase.

Received: March 7, 2024. Revised: August 5, 2024. Accepted: September 5, 2024. Available online: October 3, 2024.

1 Introduction

The *Cannabis sativa* plant has long been known for its psychotropic properties, [1]. Presently, the cannabis preparations marijuana (dried flowering tops and leaves) and hashish (compressed resin) are by far the most common substances of abuse worldwide, with an estimated 219 million subjects (4.3% of the global adult population) in 2021 who are current or previous users, according to world drug report in 2024, [2]. The plant chemistry is

complex with over 600 compounds and among them cannabinoids, terpenophenolic compounds are unique to the plant, [3], [4]. When smoked in cigarettes, water pipe, or ingested, cannabis produces a spectrum of effects, resulting principally from an action on the central nervous system such as mild euphoria or feeling high, relaxation, distorted perception of time, increased appetite, and sensory experiences, [5], [6]. These effects of cannabis are caused by its main psychoactive constituent Δ^9-tetrahydrocannabinol (Δ^9-THC) [7], which mediates its effects by acting on two types of G-protein coupled receptors [8]. These are cannabinoid receptor 1 (CB1), expressed mainly in the central nervous system in brain areas concerned with memory, cognition, motor coordination, appetite, and emotions, and CB2 receptors expressed mainly in the periphery of immune cells [8], [9]. The cannabis plant also contains other cannabinoids such as cannabinol, cannabidiol, cannabigerol, cannabidivarin, tetrahydro-cannabivarin, and cannabichromene., but these are present in small amounts and may enhance or antagonize some pharmacological effects of Δ^9-THC, [3], [8]. The effects of smoked cannabis are thus not the same as that of Δ^9-THC alone, but may represent the sum of the effects of the different ingredients and the many cannabinoids present in herbal cannabis, [10].

Cannabis is often perceived as harmless and there is a deficient awareness of the potential health consequences and other risks arising from the drug, [1], [11]. The use of cannabis, however, is associated with serious health problems, [12], [13], and these are likely to be on the rise in view of the increasing potency of cannabis in the last decade, [14], [15], [16].

Cannabis use has been linked to the development of acute cardiovascular events [17], [18]. Tachycardia is common and is due to sympathetic stimulation [17], [18], but sinus bradycardia [19], and conduction defects such as second-degree heart block [20] or even high-grade heart block, and sinus arrest [17], [21] as well as ventricular tachycardia [22] have been reported and may be caused by administering high doses of cannabis or alternatively high potency preparations. Myocardial infarction may also be precipitated by cannabis use, and result from coronary vasospam [22], [23] or thrombosis [24]. The propensity for cannabis to cause non-sustained ventricular tachycardia is higher in patients with ischaemic heart disease who are users of the herb, [25].

Therefore, the objectives of this study were to (i) look at how cannabis extract would affect electrocardiographic parameters in normal rats; (ii) examine the influence of administering cannabis in epinephrine-induced arrhythmia and myocardial damage anesthetized rat model; (ii) look for the possible modulation of the cannabis effects by the nitric oxide synthase inhibitor *NG*-nitro-L-arginine methyl ester (L-NAME).

2 Materials and Methods

2.1 Animals

For the investigation, male Sprague-Dawley rats weighing 170-180 g were employed. Rats were procured from the National Research Center's Animal House Colony. The animals were housed in environments with controlled temperature and light (20–22 °C with a 12-hour light/dark cycle), and they had unrestricted access to tap water and normal lab rodent food. The National Institutes of Health in the United States (Publication No. 85-23, amended 1996) and the Institute ethics committee's rules for using animals in experimental investigations were followed during the animal procedures.

2.2 Drugs and Chemicals

NG-nitro-L-arginine methyl ester (L-NAME) was purchased from from Sigma Chemical Co. (St. Louis, MO, U.S.A). Epinephrine was obtained from Nile Co. (Egypt). To achieve the required doses, drugs were freshly dissolved in saline immediately prior to the experiments.

2.3 Preparation of Cannabis Extract

The dried resin of *Cannabis sativa* was used to make the cannabis extract. With some modifications, the extraction process followed [26] description. In short, 10 g of the resin were ground using a mortar and pestle. The process used to decarboxylate cannabinoid acids involved putting the resin in a glass test tube, covering it with aluminum foil, and heating it for two hours in a boiling water bath. After adding 10 milliliters of analytical-grade chloroform, the mixture was left to react overnight. The cannabis substance was extracted three times. Following a filtering process using paper, the mixed fractions were gathered into a 100 ml volumetric flask. Using a gentle nitrogen stream over light-shielded ice, the filtrate was allowed to evaporate. The residue was stored at 4°C and away from light in an aluminum-covered glass flask. For the tests, the residue was again suspended

in 2 milliliters of 96% ethanol, and saline was added to bring the level up to 100 milliliters. The cannabis extract's THC content was evaluated using gas chromatography-mass spectrometry, and it was found to be 20% with only 3% cannabidiol. The study employed a dosage of cannabis extract with a Δ9-THC concentration of 20 mg/kg.

2.4 Experimental Groups

Seven equal groups of rats (n = 6 each group) were randomly allocated, and the rats were handled as follows:

Group 1 functioned as a normal control and received i.p saline treatment..

Group 2 acted as an epinephrine control, and received i.p. saline treatment 30 minutes prior to receiving an injection of epinephrine (10 µg/kg).

Group 3 was treated with i.p. cannabis (20 mg/kg).

Group 4 was treated with i.p. L-NAME (40 mg/kg).

Group 5 was treated with i.p. cannabis (20 mg/kg) 30 min prior to the i.p. injection of L-NAME.

Group 6 was treated with i.p. cannabis (20 mg/kg) 30 min prior to the i.v. injection of epinephrine.

Group 7 was treated with i.p. cannabis (20 mg/kg) 30 min prior to the i.p. injection of L-NAME and followed after 30 min by i.v. epinephrine injection.

2.5 Electrocardiography

Rats were given drugs or saline for thirty minutes, and then 10% chloral hydrate (300 mg/kg, intraperitoneal) was used for anesthesia. The ECG Powerlab module was then used to record the ECG. The latter included Lab Chart 7 software with an ECG analyzer, Powerlab/8sp, and Animal Bio-Amplifier (Australia). Following the development of a stable state, an intravenous dose of 10µg/kg of epinephrine was administered to induce arrhythmia. Each group's ECG was then recorded for 15 minutes. Over the course of 15 minutes following various treatments, the following parameters were recorded: average heart rate, RR interval, PR interval, QRS duration, QTc interval (corrected QT interval), R wave amplitude, ST segment height, number of ventricular premature beats, and duration of ventricular arrhythmia [27].

For the sake of the computations, the mean value of three 5-minute ECG recordings for each group was acquired within the first 15 minutes following an intravenous injection of epinephrine, saline, cannabis, or L-NAME alone. The number of premature ventricular beats, missing beats, and runs of ventricular tachycardia during the first 15 minutes following various drugs or after intravenous epinephrine was used to determine the presence of arrhythmia. The definition of arrhythmias was provided by [28]. A single premature ventricular complex is referred to be ventricular extrasystole, while four or more consecutive premature ventricular beats are referred to as ventricular tachycardia [28].

2.6 Cardiac Histopathology

After being immediately fixed in 10% formalin at room temperature, cardiac specimens were embedded in paraffin, treated with a standard grade of alcohol and xylol, and sectioned at 5 µm thicknesses. Hematoxylin and eosin (H & E) staining was applied to the sections so that a light microscope (Olympus CX 41 with DP12 Olympous digital camera) could be used to examine the histopathological alterations..

2.7 Statistical Analysis

For measurement variables, data are shown as mean ± SE during a 15-minute period. Tukey's multiple comparison test was conducted after a one-way analysis of variance (ANOVA) was used to compare the groups. When probability values were less than 0.05, differences were deemed statistically significant. GraphPad Prism 6 for Windows (GraphPad Prism Software Inc., San Diego, CA, USA) was utilized for the analysis.

3 Results

3.1 Changes in Electrocardiographic Parameters

The ECG alterations that are indicative of the various treatment groups are displayed in Figures 1, 2, 3, 4, 5, 6, and 7. The ECG parameter results are displayed in Tables 1 and 2, along with Figures 8 through 10.

3.1.1 Effects of a Single Dose of Cannabis in Normal Rats

The administration of a single dose of cannabis at 20 mg/kg in normal rats slowed the heart rate by 12.3% (349.3 ± 1.36 *vs*. 398.2 ± 3.10 beats/min), and increased the RR interval by 11.7% (0.172 ± 0.0007 *vs*. 0.154 ± 0.001 sec). The QTc interval was unchanged, but significantly widened QRS complex by 110.5% (0.040 ± 0.003 *vs.* 0.019 ± 0.0001 mv), and significantly depressed ST segments were observed compared with the corresponding saline control values (Table 1, Figure 1).

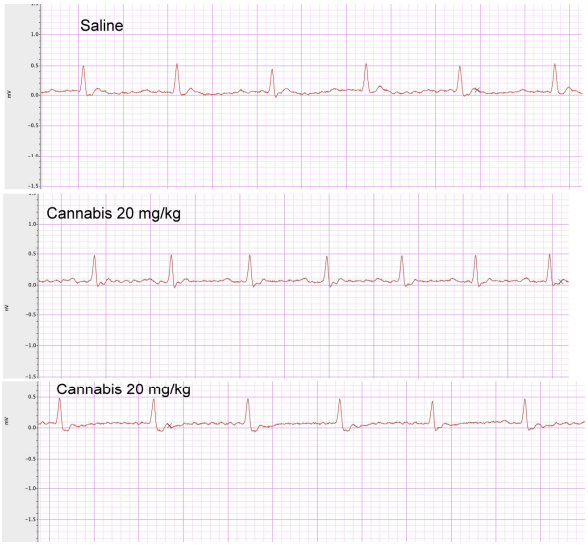

Fig. 1: Representative ECG strips of saline and cannabis only (20 mg/kg)-treated rats.

3.1.2 Effects of Epinephrine Alone

The intravenous administration of 10 µg/kg epinephrine resulted in a significant 48.1% bradycardiac response (206.8 ± 16.4 *vs.* 398.2 ± 3.10 beats/min). Additionally, there was an increase in the RR (0.067 ± 0.001 *vs.* 0.057 ±0.001 sec) and PR intervals (0.318 ± 0.021 *vs.* 0.154 ± 0.001 sec) and a decrease in the amplitude of the R wave (0.28 ± 0.048 *vs.* 0.43 ± 0.003 mv), a longer QRS duration (0.043 ± 0.005 *vs.* 0.019 ± 0.0001 sec), a shortened QTc interval (0.085 ± 0.001 *vs.* 0.111 ± 0.001 sec), and a depressed ST segment (Table 1). Following the injection of epinephrine, ventricular premature beats, polymorphic ventricular tachycardia, sinus pause, and bradycardia were seen (Figure 2, Table 2).

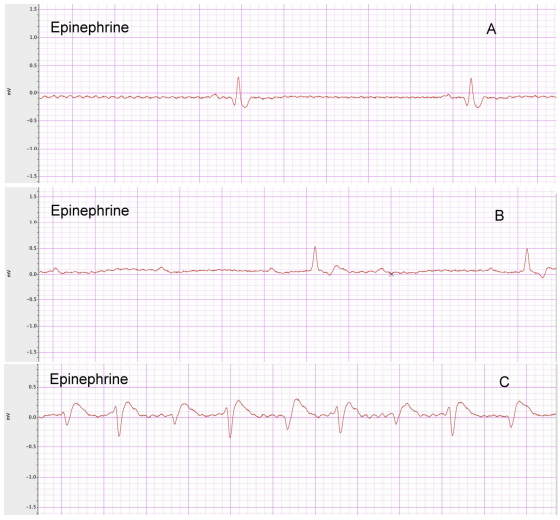

Fig. 2: Representative ECG strips of epinephrine only (10 µg/kg, i.v.)-treated rats showing (A) bradycardia and sinus pause; (B) sinus arrest; (C) polymorphic ventricular tachycardia.

3.1.3 Effects of Cannabis and Epinephrine

A single dose of cannabis at 20 mg/kg given prior to i.v. epinephrine didn't significantly change the heart rate compared with the epinephrine control (194.8 ± 19.42 *vs.* 206.8 ± 16.4 beats/min). Pretreatment with cannabis didn't significantly affect the QTc interval, but decreased the PR deceased interval by 23.2% (0.053 ± 0.002 *vs.* 0.067 ± 0.001 sec), and the QRS duration by 30% (0.030 ± 0.002 *vs.* 0.043 ± 0.005 sec). Meanwhile, R wave amplitude increased by 50% (0.42 ± 0.031 *vs.* 0.28 ± 0.048 mv) in association with significant depression of the ST segment (Table 1). Polymorphic ventricular premature beats, sinus arrest, polymorphic ventricular tachycardia, and wide QRS complex ventricular premature beats were observed (Figure. 3, Figure 4 and Figure 5). Ventricular premature beats and length of arrhythmia showed a significant increase as compared to the epinephrine control values (Table 2).

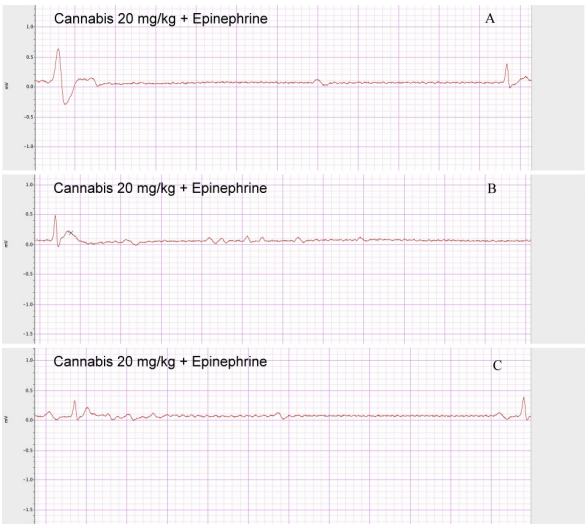

Fig. 3: Representative ECG strips of cannabis administration before i.v. epinephrine displaying ventricular premature beat in (A), sinus pause and sinus arrest in (B & C).

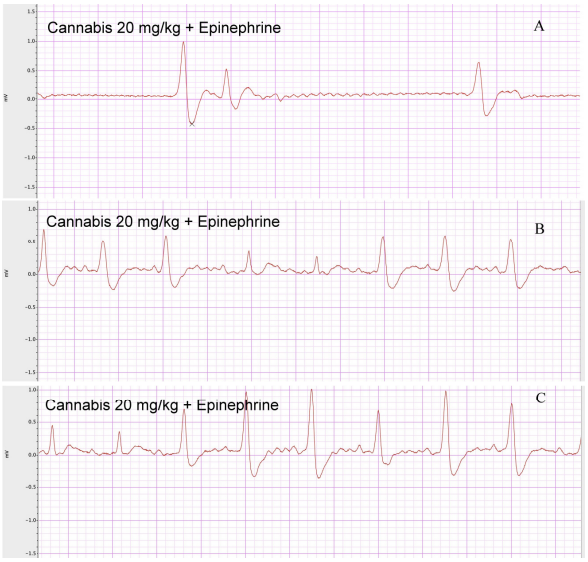

Fig. 4: Representative ECG strips of cannabis administration before the i.v. injection of epinephrine showing (A) polymorphic ventricular premature beats; (B) monomorphic premature ventricular beats; (C) polymorphic ventricular tachycardia

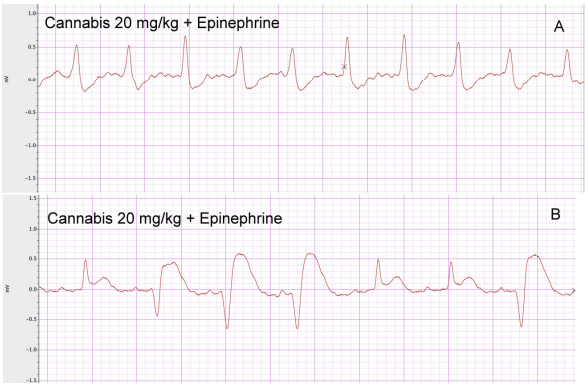

Fig. 5: Other representative ECG strips of cannabis administration prior to i.v. epinephrine showing (A) monomorphic ventricular tachycardia; (B) polymorphic ventricular premature beats and raised ST segment

3.1.4 Effects of L-NAME Alone

L-NAME caused a significant 24.5% bradycardiav response (300.6 ± 0.92 *vs.* 398.2 ± 2.98 beats/min), a significant increase in RR interval (0.199 ± 0.0006 *vs.* 0.154 ± 0.001 sec), increased PR interval (0.063 ± 0.0006 *vs*. 0.057 ± 0.001 sec), and a decrease in ST segment height (-0.037±0.006 *vs.* 0.0004±0.098 mv) (Table 1, Figure 6).

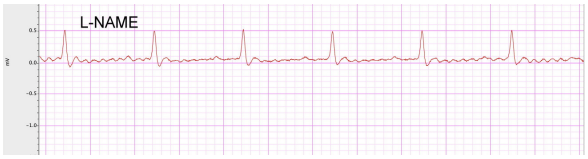

Fig. 6: Representative ECG strips of L-NAME only-treated rats

3.1.5 Effects of Cannabis, L-NAME and Epinephrine

Compared with the cannabis + epinephrine group, rats given cannabis prior to L-NAME and i.v. epinephrine exhibited significantly increased heart rate by 39.8% (272.4 ± 11.21 *vs.* 194.8 ± 19.0 beats/min), a decrease in RR interval (0.228 ± 0.011 *vs.* 0.384 ± 0.045 sec), a decrease in QRS duration by 40% (0.018 ± 0005 *vs.* 0.030 ± 0.002 sec), along with a marked decrease in R wave amplitude (0.0176 ± 0.002 *vs.* 0.42 ± 0.031 mv). These rats also showed a significantly raised ST segment (Table 1, Figure 7). The ECG showed monomorphic ventricular tachycardia, and polymorphic premature ventricular beats (Figure 7). Ventricular premature beats and length of arrhythmia showed a significant increase as compared to the cannabis/epinephrine values (Table 2).

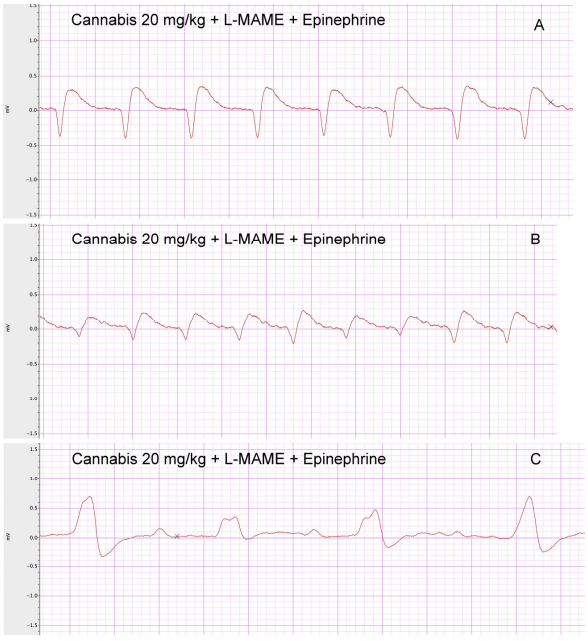

Fig. 7: Representative ECG strips of rats receiving cannabis and L-NAME prior to i.v. epinephrine showing monomorphic ventricular tachycardia (A & B), and polymorphic premature ventricular beats (C)

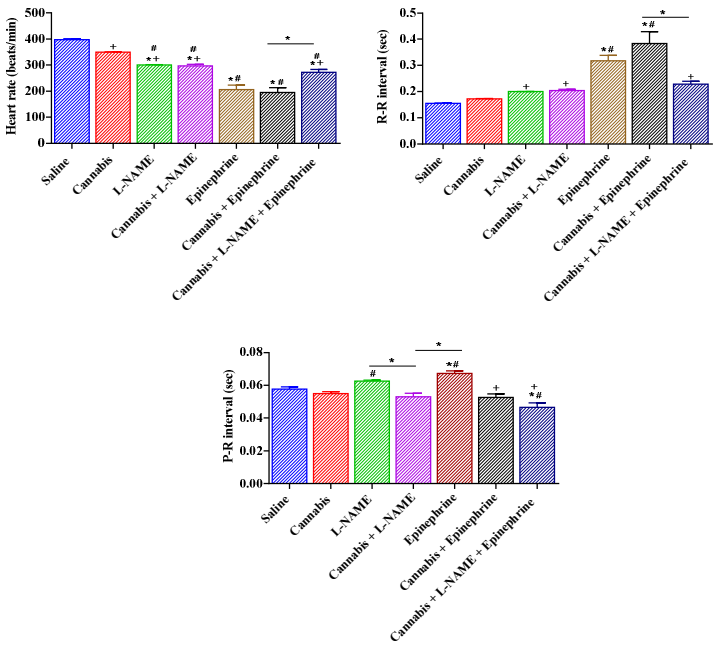

Fig. 8: Variations in the heart rate, RR interval, and PR interval among the various different groups. *: $p<0.05$: as seen in the figure, significantly different from both the normal control group and other distinct groups. A significant difference from the epinephrine control group ($p<0.05$) is indicated by the plus sign.
#: $p<0.05$: significantly different from the group that received only cannabis.

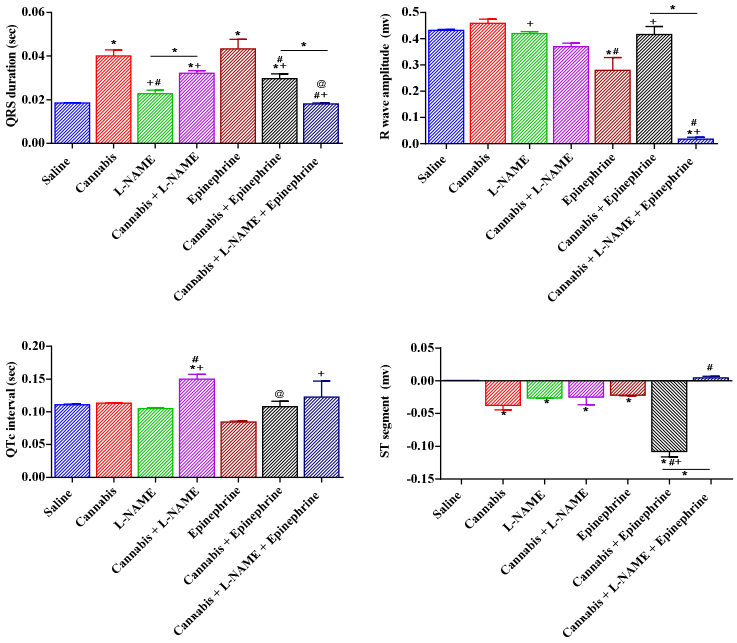

Fig. 9: shows the variations in QRS complex duration, amplitude of R wave, QTc interval, and ST segment between different treated groups. *: $p<0.05$: *: $p<0.05$: as seen in the figure, significantly different from the normal control group and other distinct groups. A significant difference from the epinephrine control group ($p<0.05$) is indicated by the plus sign. #: $p<0.05$: significantly different from the group that received only cannabis. A. significant difference ($p<0.05$) from cannabis + L-NAME group is indicated by @.

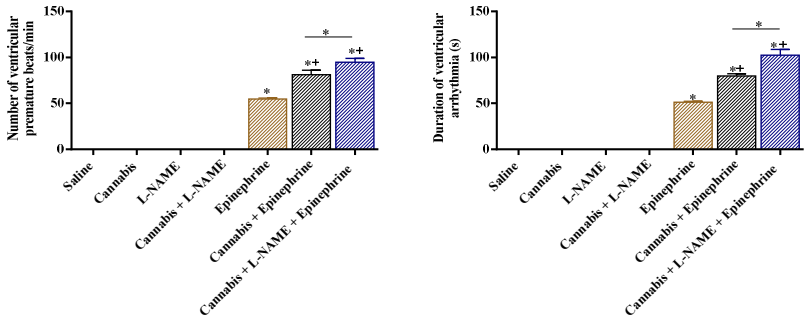

Fig. 10: shows both the number and length of arrhythmia and ventricular premature beats in various treated groups. *: p<0.05: *: p<0.05: as seen in the figure, significantly different from the normal control group and other distinct groups. A significant difference from the epinephrine control group (p<0.05) is indicated by the plus sign. #: p<0.05: denotes a significant difference from the group treated with only cannabis.

Table 1. Electrocardiographic changes after a single dose of cannabis (20 mg/kg) in normal and in epinephrine-treated rats

Parameter/ Group	Normal control	Cannabis 20 mg/kg	L-NAME	Cannabis + L-NAME	Epinephrine	Epinephrine + Cannabis	Epinephrine + Cannabis + L-NAME
Heart rate/min	398.2 ± 3.10	349.3 ± 1.36	300.6 ±0.92$^{*+\#}$	297.0 ± 6.41$^{*+\#}$	206.8 ±16.4$^{*\#}$	194.8 ± 19.42$^{*\#}$	272.4 ± 11.21$^{*+\#}$
RR interval (sec)	0.154 ± 0.001	0.172 ± 0.001	0.199 ± 0.0006^{+}	0.204± 0.004^{*+}	0.318 ± 0.021$^{*\#}$	0.384 ± 0.045$^{*\#}$	0.228 ± 0.011^{+}
PR interval (sec)	0.057 ±0.001	0.055±0.001	0.063 ± 0.0006$^{\#}$	0.053 ± 0.002	0.067 ± 0.001$^{*\#}$	0.053±0.002^{+}	0.047 ± 0.003$^{*+\#}$
QRS duration (sec)	0.019 ±0.0001	0.040±0.003*	0.023 ± 0.002$^{+\#}$	0.032 ± 0.001^{*+}	0.043±0.005*	0.030 ±0.002$^{*+\#}$	0.0181 ±0005$^{+\#@}$
QTc interval (sec)	0.111±0.001	0.113±0.0005	0.105± 0.001	0.149±0.007$^{*+\#}$	0.085±0.001	0.108±0.008$^{@}$	0.123±0.028^{+}
R wave amplitude (mv)	0.43±0.003	0.46±0.015	0.42 ± 0.008^{+}	0.37±0.013	0.28±0.048$^{*\#}$	0.42±0.031^{+}	0.018 ± 0.002$^{*+\#}$
ST segment height (mv)	0.0004±0.098	-0.037±0.006*	-0.0263±0.0004*	-0.0249±0.012*	-0.022±0.0008*	-0.0388±0.011$^{*+\#}$	0.0046 ± 0.002$^{\#}$

*The information was given as mean ± SE (n = 6). One-way ANOVA followed by Tukey's multiple comparison test was used to evaluate the data. *: p<0.05: indicated a significant difference from the normal control group. A significant difference (p<0.05)from the epinephrine control group is indicated by the plus sign. #: p<0.05: indicated a significant difference from the cannabis-only group. @: indicated a significant difference (p<0.05) from cannabis + L-NAME group.*

Table 2. Effect of a single dose of cannabis (20 mg/kg) on epinephrine-induced ventricular arrhythmia/min

Parameter/ Group	Normal control	Cannabis 20 mg/kg	L-NAME	Cannabis + L-NAME	Epinephrine	Cannabis + Epinephrine	Epinephrine + Cannabis + L-NAME
Number of ventricular premature beats	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	54.56 ± 1.25*	81.13± 2.03^{*+}	94.63 ± 1.79$^{*+\#}$
Duration of arrhythmia (sec)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	51.21±1.02*	79.96 ± 0.87^{*+}	102.3 ± 2.51$^{*+\#}$

*The data are expressed as mean ± SE.). One-way ANOVA followed by Tukey's multiple comparison test was used to evaluate the data. *: p<0.05: indicated a significant difference from the normal control group. A significant difference (p<0.05)from the epinephrine control group is indicated by the plus sign. #: p<0.05: indicates a significant difference from the cannabis + epinephrine group.*

3.2 Histopathological Results

Sections of the saline control rats' heart muscle were examined. It displayed striated myocardium, which was arranged in a linear array that branches and anatomizes in a certain way to resemble a sheet. Intercalated discs bind the fibers of the heart muscle together. A thin layer of connective tissue with clearly visible myocardial blood capillaries separates the heart muscle fibers (Figure 11A). The histological changes in the cardiac muscle of rats treated with epinephrine revealed wavy appearing cardiomyocytes, focal degeneration of muscle fibers, coagulative necrosis. Some nuclei appeared pyknotic and/or hypertrophied and flattened, and

others appeared pale and there was the disappearance of nuclei (Figure 11B). Rats treated with L-NAME and cannabis showed the normal architecture of the cardiac muscle (Figure 11C). Cardiac sections of rats treated with cannabis and epinephrine showed the cardiac muscle still suffer from pathological changes in the form of disorganized muscle fibers, scattered hyalinized fibers, pyknotic nuclei, separation of myofibrils and edema (Figure 11D). Cardiac sections of rats treated with cannabis, L-NAME, and epinephrine showed disarrangement of cardiac muscle fibers, congestion of blood vessels and few area of hyalinization fiber. Also, vacuolation, pyknotic nuclei and inflammatory cell invasion in muscle fibers were still present (Figure 11E).

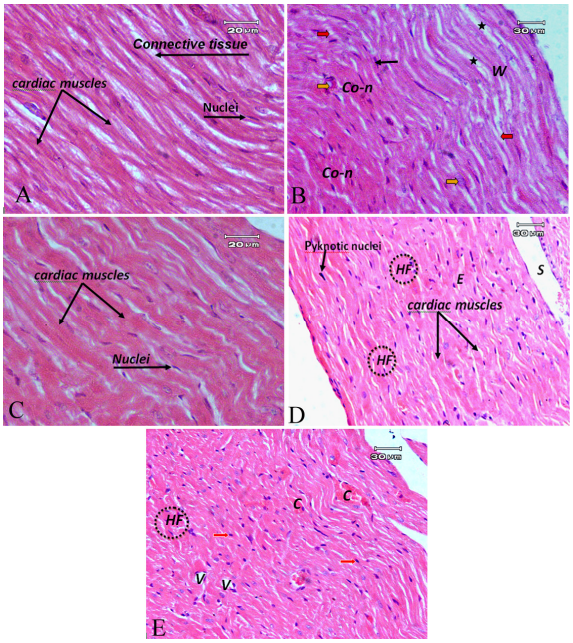

Fig. 11: Representative photomicrographs of the rats' heart muscles following treatment with (A) saline, which displays cardiac muscle fibers arranged longitudinally and regularly striated, with central, vesicular, and oval nuclei (black arrow); **(B)** Epinephrine revealing focal coagulative necrosis (co-n), few areas of wavy cardiac muscle fibers (W), focal area of degeneration (red arrow), the nuclei appeared dense, shrunken (black arrow) and others appeared pale and there was disappearance of nuclei (orange arrow); **(C)** L-NAME and cannabis showing the normal architecture of the muscle fibers and nuclei; **(D)** Cannabis and epinephrine showing disorganized muscle fibers, scattered hyalinized fibers (HF), pyknotic nuclei, separation of myofibrils (S) and edema (E); **(E)** L-NAME, cannabis and epinephrine showing disarrangement of cardiac muscle fibers, congestion of blood vessels (C), few areas of hyalinized fibers (HF), cytoplasmic vacuolation (V), pyknotic nuclei, and inflammatory cells.

4 Discussion

The present study investigated the effects of a single dose of cannabis (20 mg/kg) on ECG parameters in the rat. We tested the effect of cannabis on arrhythmic activity and cardiac muscle injury in the epinephrine-induced arrhythmia model. We also evaluated the possible modulation of nitric oxide synthase inhibition using L-NAME on the response to cannabis. The results of this study showed that cannabis itself slowed the heart rate, and significantly widened QRS complex. Cannabis showed no effect on the QTc interval, but significantly depressed ST segment when compared with the saline control. Apart from slowing the heart rate, cannabis alone didn't cause ventricular arrhythmia. However, cannabis administration significantly increased the number of ventricular premature beats, ventricular tachycardia, and duration of rhythm abnormalities caused by i.v. epinephrine. Nitric oxide synthase inhibition with L-NAME worsened the ventricular arrhythmia encountered in the cannabis/epinephrine-treated rats. These observations indicate that cannabis exerts detrimental effects on epinephrine-induced ventricular arrhythmia. The study also provides further support for the anti-arrhythmogenic and cardioprotective actions of endogenously released nitric oxide.

The effects of herbal cannabis or its principal psychoactive constituent Δ^9-THC on cardiac electrophysiology and their propensity to provoke cardiac arrhythmia have been examined in humans and laboratory animals as well as in vitro. When given i.v. in man, Δ^9-THC (25 µg/kg) caused a marked increase in heart rate, enhancement of sinus automaticity, and facilitation of both sinoatrial and A-V nodal conduction, [29], [30]. Other studies reported S-T segment, T-wave changes, and premature ventricular contractions following repeated ingestion of 300 µg/kg of Δ^9-THC, [31]. Users of marijuana had more supraventricular tachycardia, premature atrial contractions, and non-sustained ventricular tachycardia compared with never-users, [32]. Marked sinus bradycardia, second-and third-degree atrioventricular heart block, and sinus arrest were also reported after high doses of smoked herbal cannabis, [17], [19], [20], [21]. In contrast, in isolated rat hearts *in vitro*, perfusion with Δ^9-THC caused a slowing of heart rate that was unresponsive to atropine–propranolol, [33]. In

animals, Δ^9-THC caused bradycardia and hypotension, [18]. It has been suggested that the effects of cannabis or Δ^9-THC on the heart are largely mediated by an action on the autonomic nervous system and are dose-dependent in that that low to moderate doses are associated with tachycardia and increased arterial blood pressure, while high doses have opposite effects resulting in bradycardia, sinus arrest, and serious ventricular tachyarrhythmia, [17], [30], [34].

Our present study suggests that a high dose of cannabis by itself does not provoke cardiac arrhythmia in normal rats. In contrast, cannabis enhanced the arrhythmic action of i.v. epinephrine. The mechanisms underlying this pro-arrhythmogenic action of cannabis may include an effect on the sympatho-parasympathetic balance in the cardiac conduction system, [18], [30]. It is also possible that a vasoconstriction effect and slowing of coronary blood flow [22] with consequent changes in coronary microcirculation and impairment of cellular energy metabolism play a role.

The present study also examined the effect of inhibition of nitric oxide synthases using L-NAME on the cannabis-induced ECG and histological changes. Nitric oxide produced from L-arginine via the action of nitric oxide synthases is important in the regulation of vascular tone and coronary blood flow, cardiac contractility, and excitability, [35]. Nitric oxide released from the vascular endothelium, cardiac myocytes, and nerves may function as an endogenous anti-arrhythmogenic and cardioprotectant *via* mechanisms such as maintaining synchronous beating, conductivity, and vascular tone, besides a decrease in cellular oxidative stress, and suppression of sympathetic nerve activity, [35], [36], [37]. Our results showed that L-NAME resulted in significant bradycardia and increased RR interval which is in accordance with previous studies, [38]. L-NAME given alone or after cannabis, apart from sinus bradycardia, did not cause ventricular arrhythmia. However, rats treated with cannabis prior to L-NAME and i.v. epinephrine exhibited a significantly increased number of ventricular premature beats and duration of arrhythmia compared with the cannabis/epinephrine or epinephrine-only treated animals. Inhibition of endogenous nitric oxide production by L-NAME thus resulted in worsening of the epinephrine or cannabis/epinephrine-induced arrhythmia. On the other hand, disarrangement of cardiac muscle fibers, and a few areas of hyalinized fibers, pyknotic nuclei the inflammatory cells were seen in cardiac sections from the cannabis, L-NAME and epinephrine groups.

5 Conclusion

In summary, the present study provided the evidence that acute administration of cannabis in high dose caused slowing of heart rate, and ST segment changes suggestive of myocardial ischaemia. Cannabis also increased ventricular arrhythmia induced by epinephrine. The arrhythmia caused by cannabis/epinephrine administration was enhanced by inhibiting nitric oxide synthesis with L-NAME. The cardiac histology after epinephrine, cannabis/epinephrine or cannabis/L-NAME/epinephrine revealed marked structural changes in the form of disorganization and degeneration of muscle fibers. The study has important clinical applications in that it indicates a propensity for developing serious ventricular arrhythmias, heart block or sinus arrest following epinephrine administration in a cannabis user. Future studies on the effects of long-term cannabis are required.

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Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

Omar Abdel-Salam, Amany Sleem and , Marawan Abd El Baset designed the study. Marwan Abd El Baset perfomed the ECG measurements. The histological studies and their interpretation were dine by Fatma Morsy. Omar Abdel-Salam prepared the manuscript. Omar Abdel-Salam, Marawan Abd El Baset, Amany Sleem and Fatma Morsy approved to the final version of the manuscript.

Sources of Funding for Research Presented in a Scientific Article or Scientific Article Itself

No funding was received for conducting this study.

Conflict of Interest
The authors declare no conflicts of interest