Role of Cannabinoid Receptor 1 Antagonist (Rimonabant) in Cardiac Dysfunctions in Experimentally Induced Schizophrenia in Rats

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Abstract

Background and objectives: Clozapine (CLZ) is a successful medication for treating resistant, incurable schizophrenia. However, due to its cardio toxic effect, worries regarding its safety had grown. The current study examines how the CB1 receptor antagonist rimonabant influences the cardio toxicity brought on by CLZ and the control of schizophrenia that CLZ provides.

Methods: 48 adult male Wistar rats were used in the study, which lasted 5 weeks, and were separated into 6 groups: negative control, rimonabant solvent, and rimonabant control (3 mg/kg/d). The ketamine schizophrenia model (30 mg/kg), CLZ treatment (25 mg/kg/day) and rimonabant and CLZ treated. At the end of the study, cardiac functions were recorded. Morris maze test and forced swimming test were conducted to assess schizophrenia control and after sacrifice sera and hearts were examined for CLZ-induced toxic effects and brains were examined for dopamine and serotonin.

Results: CLZ caused a significant increase in serum and cardiac inflammatory markers, marked electrophysiological disturbances and myocardial inflammation and fibrosis scores p value <0.001. Rimonabant significantly attenuated the histopathological and biochemical manifestations of myocarditis. Rimonabant also significantly improve Morris maze test and forced swimming test results beyond those obtained with CLZ alone. Dopamine and serotonin levels were raised with co-administration of both drugs. Conclusion Rimonabant has protective actions against CLZ-induced myocarditis with positive additive effect on schizophrenia control.

Keywords: CLZ, schizophrenia, cannabinoid antagonist, myocarditis, rimonabant, cardiac toxicity

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INTRODUCTION

One of the most severe, life-threatening mental diseases is schizophrenia.[1] About 1% of the world's population is impacted by it.[2] it develops early (15–35 years), lasts long time, and causes permanent disability. [3] Typical or atypical antipsychotic medicines serve as the mainstay of treatment [4] Clozapine (CLZ), a tricyclic benzodiazepine derivative, was the first atypical antipsychotic drug and is now the first line of treatment for those with refractory schizophrenia. [5] CLZ is a unique antagonist at both dopamine and serotonin receptors, It has greater affinity for dopamine D4 than dopamine D2 receptor resulting in reduction in the negative symptoms and extrapyramidal side effects. [6] It also remains the only agent approved for reducing the suicide behaviors in schizophrenia patients. [7] However a wide variety of immunological and cardiovascular side effects had restricted its usage. [8] [9, 10] More commonly, metabolic complications. [11] Cardiovascular pathologies are a main cause of death in schizophrenia patients which cause a 3-4 fold increased risk of premature death. [12] CLZ most probably causes myocarditis, beside reported cases of dilated cardiomyopathy, pericarditis and sudden cardiac death. [13] Although the exact

mechanisms underlying CLZ's cardio toxicity are still unknown, a hypersensitive response and elevated catecholamine levels can account for it. [14]. a rise in oxidative stress and the generation of pro-inflammatory cytokines.[15] captopril and beta blockers may attenuate the cardio toxicity of CLZ, according to several clinical investigations.[16] A unique lipid signaling system, the endocannabinoid system (ECS), has been linked to a number of physiological functions. Endocannabinoids (eCBs), cannabinoid receptors (CBRs), and enzymes that biosynthesize and degrade eCBs make up this system. [17] Cannabinoid 1 receptors, CB1R, are mainly located in the mammalian brain. But it plays also role in the cardiovascular risk factors in obesity/metabolic syndrome. [18] Interestingly, pharmacologic inhibition of CB1R showed protective effects against doxorubicin-induced cardio-toxicity. [19] preliminary study suggested that pharmacological inhibition of CB1R was beneficial in CLZ-induced cardiotoxicity.[20] Rimonabant's use in schizophrenia is controversial, nonetheless, as some publications have noted negative psychiatric side effects. As a result, the current study's objectives were to examine how the cannabinoid receptor 1 antagonist rimonabant affected the cardiac dysfunction brought on by CLZ and to define how this pharmacological combination affected schizophrenia. This offers a rare chance to apply "reverse translational medicine, " which is the process of going from preclinical models to clinical trials.

MATERIALS AND METHODS

Animals used

Male wistar strain rats (170-200 g), purchased from The Biological Research Unit, Faculty of Medicine, Cairo University, Egypt, were used in this study.

Animals were kept under standardized conditions (temperature 25±2˚C, 12: 12 h light-dark cycle and 50±5% relative humidity). With free access to a balanced food. Water was offered in separate clean containers. Before starting the experiments, animals were left 14-day of adaptation period to the new environment and to ensure competability. All the experiments were carried out during the light period (08: 00-16: 00 h). The animals‟ treatment protocol was in accordance with the Guidance of The Institutional Animal Care and Use Committee (IACUC), Cairo University. (I ACUC Protocol Number: CU III f 88/19)

Chemicals used

a) Rimonabant powder 5-(4-chlorophenyl)-1-(2, 4-dichlorophenyl)-4-methyl-N-piperidin-1-ylpyrazole-3-carboxamide (MedChem-Express LLC pharmaceutical company, USA) was dissolved in the following solvent: 30% PEG400 (polyethylene glycol 400) + 0.5%TW 80(Tween 80) + 5% (PG + 64.5% distilled water. Rocchio, Neilsen, Everett, & Bothun, 2017) It was administered to rats by means of intra peritoneal (IP) injection.[21]

b) CLZ powder (8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo [b, e] [1, 4]-diazepine) from Sigma-Aldrich, Cairo, Egypt in the form of yellow powder that was dissolved in distilled water.It was administered by means of oral gavage. [22]

c) Ketamine solution (Sigma Pharmaceuticals, Egypt): Rats received 30 mg/kg/day intramuscular for consecutive 5 days for induction of schizophrenia followed by 1 week period of ketamine washout. [23] [24]

In the current study, CLZ dose of 25 mg/kg was used, which was comparable to the clinical dosage following the conversion of humans to rats. In the beginning, we also combined 15 mg/kg/day dose with 25 mg/kg/day, and we discovered that the dose of 15 mg/kg did not cause any notable biochemical or histological abnormalities in comparison to rats receiving a negative control. Rimonabant, 10 mg/kg/day (i.p.) dose produced considerable weight loss in obese mice. [21] We tested the lower dose of 3 mg/kg.

Experimental protocol

Total 48 rats were divided randomly in 2 main models 24 rats in each non-ketamine treated non-schizophrenic model (n=8 per group) and ketamine treated schizophrenic model (n=8 per group). Experiments were conducted over a period of 5 weeks.

A) Non-ketamine treated (control rats)

Negative control group: received saline by IP injection during the first week, starting from the beginning of the third week they received 1ml/kg/day saline for two weeks.

Solvent control group: rats received a solvent of rimonabant with the following component: 30%PEG 400 (polyethylene glycol 400) + 0.5% TW 80(Tween 80) + 5% PG + 64.5% distilled water by IP injection at volume 0.25 ml/kg/day for the last two weeks of the experiments.

Rimonbant control group: received intra peritoneal rimonabant at daily doses of 3 mg/kg for the last 2 weeks.

B) Ketamine-treated (schizophrenic rats)

Ketamine control group: Rats received Ketamine (30 mg/kg) by IP injection daily for 5 days, for induction of schizophrenia followed by a wash out period of 1 week.

CLZ treated group: Rats were subjected to ketamine induction of schizophrenia for 5 days and a wash out period of 1 week then were given daily for 2 weeks: intra peritoneal saline followed after one hour by 25 mg/kg CLZ orally.

Rimonbant and CLZ treated group: Those animals were treated exactly as performed for Group 5 but rimonabant was injected intra peritoneal at daily doses of 3 mg/kg 1 hour before CLZ administration and for the whole period of CLZ treatment. [25]

Animals were weighed at the start and at the end of the experiments using light sensor. Rats were given ether in oxygen anesthesia after the end of the experiment, and hemodynamic tests were then performed. Blood samples were taken from the retro orbital plexuses after the hemodynamic tests. Animals were then scarified and a piece of filter paper was used to blot the hearts before they were immediately weighed and the heart/body weight ratio (HW/BW) was computed. After that, hearts were split in half mid-ventricular, with one half used for biochemical tests. The second-half ventricles were employed for the histological examination.

Hemodynamic studies

- Non-invasive blood pressure monitors (LE 5001, LETICA scientific Instruments, Spain) was used to record blood pressure from the tail of conscious rats by the tail cuff technique
- Electrocardiogram (ECG) recordings: ECG was recorded at the end of the study for evaluation of possible presence of left ventricular hypertrophy (e.g. tall R wave), ischemic changes (e.g. ST segment depression, T-wave inversion) or arrhythmias (e.g. extra systoles, QT prolongation).

Behavioral studies

- Morris water maze test.

The test consists of a 5-day acquisition phase and a one-day probe trial phase. [26] [27] [28]

- Forced Swimming Test (FST).

The original protocol of FST is dependent on the idea that rodents after the first escape movements develop immobility if placed in a non-escapable cylinder of water (pretest). Then when they were replaced in it 24 hours later; they reassume this posture rapidly (test). [29] [30] The immobile position reflects the presence of depressive behavior (depression) that prevents the animal activity to handle stress. [31]

Biochemical studies

24 hours after the last treatment, blood samples were collected into clean test tubes without anticoagulant, from the retro orbital plexuses. The sera were then separated and kept frozen at 20 C for estimation of Creatine Kinase (CK), Lactate Dehydrogenase (LDH) and Aspartate aminotransferase (AST) levels.

CK level as (U/L) was estimated in serum according to the method of Bishop et al. [32] using diagnostic kit from Bio diagnostic Co. (Cairo-Egypt). LDH level was determined using diagnostic kit provided from Bio diagnostic Co. (Cairo-Egypt) and was calculated as (U/L) according to the method of Whitaker [33] The serum concentration of AST was measured by aspartate aminotransferase (AST) ELISA Kit (Catalogue Number: SL0311Hu) provided by Sun Long Biotech Co., LTD.

2-Heart homogenate

After scarification, the supernatant of heart tissues were used for:

- Estimation and TNF-α and IL6

TNF-a was measured using TNF-α immunoassay kit (Catalog Number: SEKH-0047) provided by Solarbio life sciences (China) by enzyme-linked immunosorbent assay (ELISA) technique and as previously described. [34] The concentration of IL-6 in heart was measured (by the same technique as TNF-α and according to manufacturer's instructions.) Using IL-6 immunoassay kit (Catalog Number: SEKH-0013) provided by Solarbio life sciences (China).

- Estimation of Lipid Peroxidation MDA

Using Malondialdehyde (MDA) ELISA Kit (Catalogue Number: SL1135Hu). The kit was provided by SunLong Biotech Co., LTD (China).[22]

Estimation of brain dopamine and serotonin levels

The brain hippocampal tissues were excised, weighed and homogenized in (PBS) (pH 7.4) then the supernatant was separated to be used for determination of: Dopamine level using dopamine (DA) ELISA Kit (Catalogue Number: SL1926Hu) provided by SunLong Biotech Co., LTD. China. Ser-

otonin level using serotonin ELISA Kit (Catalogue Number: SL1570Hu) provided by SunLong Biotech Co., LTD (China).

Histopathological analysis

For histological analyses to identify inflammatory infiltrates, heart tissues were fixed in a 10% neutral formalin solution, embedded in paraffin, sectioned at a thickness of 4 m, and stained with haematoxylin and eosin (HE). In the present study, a recruitment of at least 10 inflammatory cells was needed to define myocarditis. Based on the level of cellular infiltration, a 5-point scale with a range of 0 to 4+ was used to score the inflammatory infiltrates.[35] A score of 0 meant there were no lesions present or suspected to exist in each category. A score of 1+ meant that the myocardial lesions were confined to a small region. While a score of 4+ indicated the presence of coalescent and widespread lesions across the whole area of cardiac tissue tested, scores of 2+ to 3+ suggested moderate severity with multifocal lesions. The evaluation of fibrotic lesions was done using Masson's Trichrome staining. After Masson's Trichrome staining, the acquired heart tissues clearly displayed red myocytes and blue collagen fibers. By dividing the blue-stained regions by the total myocardial tissue area, the amount of total fibrosis was calculated and estimated, and the severity of the fibrosis was classified as none, minimal, mild, moderate, or severe. [36]

Statistical Analysis

Data were coded and entered using the statistical package SPSS version 21.0. Percentage change in body weight was calculated as follows: [(final weight – baseline weight) / baseline weight]. Changes in Water Maze test were calculated as follows: final value – baseline value; so positive values of change reflected an increase and negative ones reflected a decrease in time. Checking data distribution revealed that most of the group values were non-Normally distributed and so it was decided to use non-parametric tests to derive inferences throughout the data analysis. So for descriptive purposes the median and the quartiles were used. Comparisons among groups within each model were performed by the Kruskal Walis test because each model contained more than two groups. When preliminary analysis showed a statistically significant difference among the groups (judged by presence of a p value < 0.05). Relevant pairwise comparisons were done by Mann Whitney test. Judgments here were mostly conservative, considering multiple comparisons that risk inflation of the Type 1 error probability. So the conventional alpha value was divided by the number of comparisons performed (Bonferroni correction) within a model to guide decisions about statistical significance. Al pairwise comparisons were two-sided. For exploring associations among different measure parameters, Spearman correlation was used, utilizing values from all groups in both models. Scatter plots were constructed to explore the direction of any possible correlation and amtrix of bivariate correlations was built.

RESULTS

Body weight and heart/body weight ratio

Animals in the negative control, solvent control and ketamine treated groups gained an average of 20% above basal body weight in the period of 4 weeks. Those taking clozapine gained more weight, while rimonabant treatment prevented the body weight gain induced by clozapine in rimonabant/clozapine group. Rimonabant control animals showed significant weight loss. ($p =< 0.001$) There was no statistically significant deference regarding heart/body weight ratio among the studied groups.

- **Electrocardiogram recordings**

Ketamine treated animals displayed a shortening in the PR interval as compared to the negative control group. The CLZ treated group showed significant short PR, prolonged QTc intervals, higher R wave amplitude, flattening of the T wave and ST segment depression as compared to the negative control and ketamine treated groups. Combined rimonabant and CLZ treatments produced ECG parameter values that lay midpoint between those of CLZ-treated and those of the control groups. Table 1

Table 1: Electrocardiogram recordings

	Non-ketamine treated			Ketamine treated			P value
	Negative control (n=8)	**Solvent control** (n=8)	**Rimonabant control** (n=8)	**Ketamine treated** (n=8)	**CLZ treated** (n=8)	**Rimonabant /CLZ treated** (n=8)	
Heart Rate (BPM) Median (Quartiles)	213 (197, 234)	238 (226, 239)	180 (163, 212)	244 (220, 270)	262 (246, 270)	223 [d] (209, 239)	0.001*
PR Interval (s) Median (Quartiles)	0.05 (0.04, 0.057)	0.043 (0.03, 0.047)	0.05 (0.04, 0.17)	0.04[a] (0.036, 0.04)	0.047 [a, c] (0.04, 0.055)	0.041 [a, b] (0.038, 0.046)	0.007*
QTc (s) Median (Quartiles)	0.14 (0.08, 0.17)	0.13 (0.08, 0.17)	0.15 (0.08, 0.19)	0.13 (0.13, 0.15)	0.2 [a, c] (0.19, 0.2)	0.15 [d] (0.12, 0.16)	0.019*
R wave (mv) Median (Quartiles)	0.3 (0.2, 0.37)	0.26 (0.2, 0.36)	0.33 (0.2, 0.4)	0.27 (0.13, 0.4)	0.63 [a, c] (0.44, 0.84)	0.4 [d] (0.22, 0.46)	0.006*
T wave (mv) Median (Quartiles)	0.173 (0.13, 0.26)	0.17 (0.13, 0.26)	0.17 (0.13, 0.26)	0.26 (0.19, 0.23)	0.08 [a, c] (0.04, 0.09)	0.19 [d] (0.15, 0.2)	0.009*
ST (mV) Median (Quartiles)	0.04 (0.04, 0.09)	0.04 (0.03, 0.06)	0.043 (0.04, 0.09)	0.05 (0.03, 0.08)	-0.08 [a, c] (-0.13, -0.04)	-0.035 [a, c, d] (-0.05, -0.029)	<0.001*

BPM=beats per minute; QTc=corrected QT interval; * =statistically significant;
[a] = significantly different from negative control; [b] = significantly different from solvent control;
[c] = significantly different from ketamine control;
[d] = significantly different from the CLZ-treated model

Biochemical analysis

Biochemical parameters measured are demonstrated in Table 2. There were significantly higher levels of serum CK, LDH and AST in the CLZ-treated group. The levels of CK, LDH and AST were significantly lower in animals treated with rimonabant and CLZ as compared to those treated with CLZ alone with no significant differences as compared to the control groups. (CK; p=0.002, LDH; p=0, 005, AST; p=0.005). Animals treated with CLZ also showed highly significant increase of TNFα, IL6 and MDA levels compared to negative control and ketamine treated animals. Rimonabant treatment produced highly significant reduction in TNFα and MDA levels with p values of 0.002 and and<0.001, respectively. IL6, however, was significantly lower compared to CLZ treated group with no significant difference from control groups values (p=0.003).

Table 2: Biochemical parameters measured in serum (CK, LDH, and AST) and heart (TNFα, IL6, MDA)

	Non-ketamine treated			Ketamine treated			P value
	Negative control(n=8)	**Solvent control** (n=8)	**Rimonabant control** (n=8)	**Ketamine treated** (n=8)	**CLZ treated**(n=8)	**Rimonabant /CLZ treated**(n=8)	

CK (U/L) Median (Quartiles)	279 (231, 279)	359 (266, 465)	272 (256, 278)	308 (270, 401)	879 [a, c] (770, 892)	354 [d] (278, 451)	0.002*
LDH(U/L) Median (Quartiles)	279 (263, 281)	295 (237, 320)	330 (312, 358)	354 (244, 404)	498 [a, c] (440, 564)	297 [d] (229, 340)	0.005*
AST(U/L) Median (Quartiles)	92 (87, 92)	117 (98, 124)	116 (107, 121)	121 (110, 127)	149 [a, c] (139, 152)	112 [d] (98, 130)	0.005*
TNFα (pg./mg) Median (Quartiles)	75 (67, 75)	95 (89, 101)	95 (82, 112)	105 a (96- 115)	190 a, c (183, 216)	100 c, d (90, 108)	0.002*
IL6 (pg./mg) Median (Quartiles)	28 (22, 28)	32 (27, 39)	31 (28, 35)	29 (26, 32)	73 a, c (60, 84)	35 d (30, 50)	0.003*
MDA (nmol/g) Median (Quartiles)	8.5 (8.4, 8.5)	10.2 (9.6, 11.7)	9.8 (9.1, 10.5)	11a (10, 13)	20 a, c (18, 23)	9 c, d (8, 9.6)	<0.001*

CK=Creatine kinase; LDH= Lactic acid dehydrogenase; AST= Aspartic transaminase; TNFα= Tumor necrosis factor alpha; IL6= Interleukin 6; MDA= Malondialdehyde; * =statistically significant;
[a] = significantly different from negative control; [b] = significantly different from solvent control;
[c] = significantly different from ketamine control; [d] = significantly different from the CLZ-treated

- **Histopathological analysis of heart tissue:**

No significant variation was noted among all the control groups scores (0-1). The ketamine treated group showed higher fibrosis scores (1-2) as compared to those of the negative control group.

The CLZ-treated group showed evidence of significant inflammation with inflammatory cell recruitment with score (2.7-4) and moderate to marked fibrosis of heart tissues. Average scores of inflammation and fibrosis were significantly lower when rimonabant was combined with CLZ. Inflammation and fibrosis markers were however still detectable and were significantly higher as compared to those of the control groups. P value <0.001 for both of them. Table 3

The histological findings in the heart tissues of the control non-ketamine treated groups were within the normal histological structures, with elongated cardiomyo-fibers, central spherical single nuclei, and a light eosinophilic cytoplasm except for solvent treated animals which showed mild inflammatory changes and minimal fibrotic lesions. Histological sections from CLZ treated animals' revealed disorganization and degeneration of the myocardial tissues and exhibited moderate vascular congestion and perivascular inflammation with inter-muscular edema which was reliable with myocarditis. Fibrotic lesions were found in the left ventricles. Figure 1

Table 3: Inflammation and fibrosis analysis score of heart tissue

	Non-ketamine treated			**Ketamine treated**			**P value**
	Negative control(n=8)	**Solvent control(n=68)**	**Rimonabant control(n=8)**	**Ketamine treated(n=6)**	**ClZ treated(n=8)**	**Rimonabant /CLZ treat-**	

						ed(n=8)	
Inflammation score Median (Quartiles)	0	0 (0, 1)	0	0 (0, 1)	3 [a, c] (2.7, 4)	1 [a, d] (0.75, 2)	<0.001 *
Fibrosis Median (Quartiles)	0	1 (1, 2)	0	1 [a] (1, 2)	5.5 [a, c] (4.7, 7.3)	3 [a, c, d] (2, 3.3)	<0.001 *

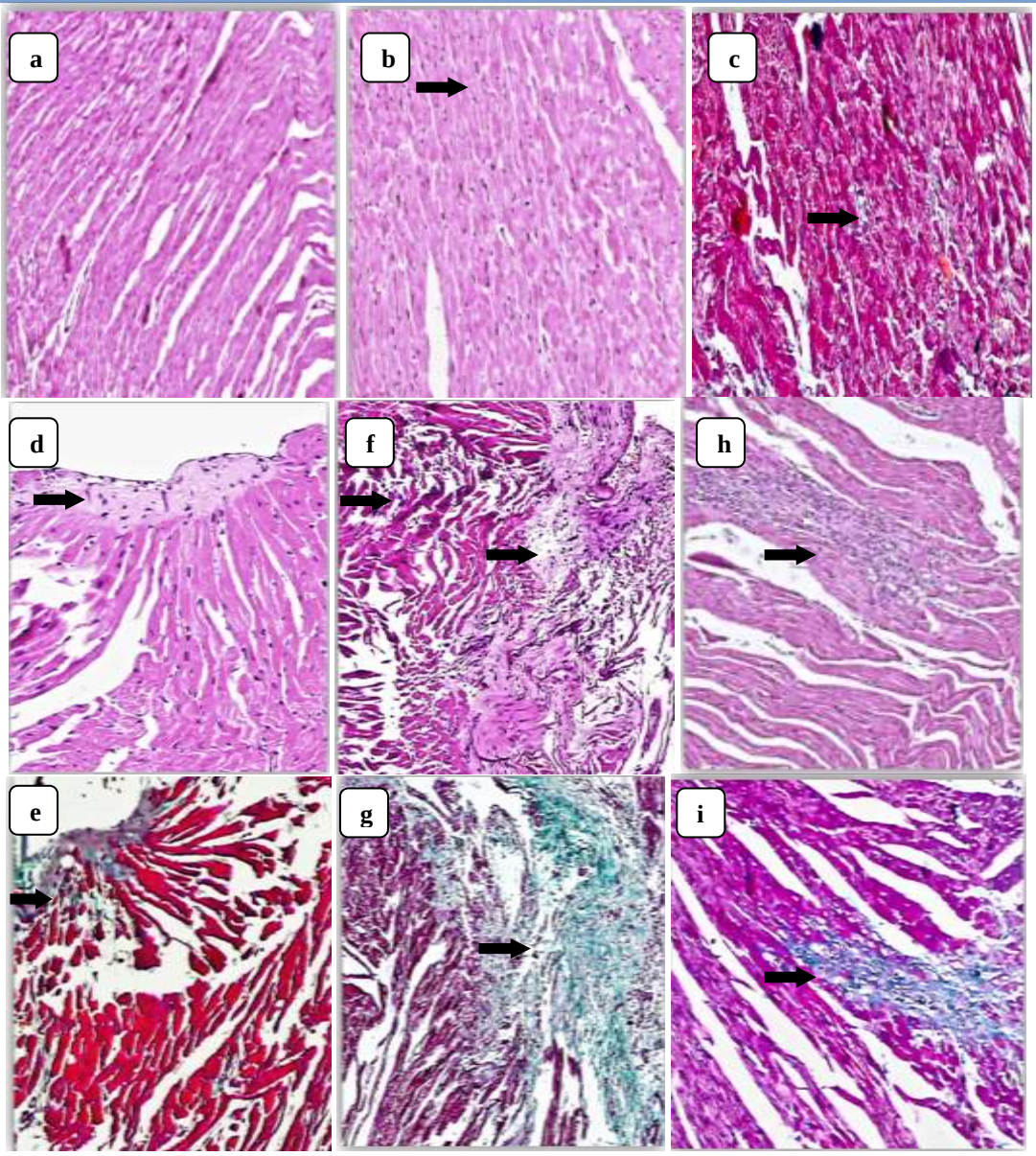

Figure 1: (a) Myocardium of rats of the control and rimonabant-treated groups showing normal branching anastomosing myocardial fibers with acidophilic cytoplasm and centrally located, large, pale, vesicular nuclei. **(b)** Myocardium of rats of the solvent control group showing minimal inflammation and fibrosis traces in figure **(c)** (arrows). **(d)** Myocardium of rats of the ketamine treated group showing minimal inflammation and fibrosis traces in figure **(e)** (arrows). **(f)** Myocardium or rats of CLZ-treated group showing congestion, inflammation, and edema and fibrosis in figure **(g)**. (arrows). **(h, i)** Myocardium of rats of CLZ/rimonabant - treated group showing minimal inflammation and edema and near normal architecture in figure (h) and minimal fibrosis in figure (i). (arrows) CLZ: clozapine.

- **<u>Behavioral tests analysis and biochemical analysis of brain tissue</u>**

Table 4 summarizes MT test results. No significant differences were noted at base line. Comparisons of the percentage of change in test values showed that ketamine treatment produced significant memory loss which was partially corrected by CLZ with (p = 0.008).Combined rimonabant/CLZ treatment produced highly significant increase in memory retention and even showed improvement of cognitive function as compared to CLZ only treated animals. Regarding forced swimming test, Animals in the ketamine group showed significant reduction in active swimming time as compared to the negative control group, while CLZ treatment induced significant improvements in active swimming time compared to the ketamine group. This time was significantly higher when rimonabant was combined with CLZ treatment. (p=0.001).

Ketamine treated animals showed significantly higher level of dopamine and serotonin. CLZ treatment significantly reduced levels of those transmitters with p= 0.001 for both. The significant reductions were maintained when rimonabant was combined with CLZ for ketamine-treated rats. Average serotonin level, however, was significantly higher in CLZ/rimonabant group compared to the CLZ group.

Table 4: Behavioral tests and Brain tissue Serotonin, Dopamine measurements

	Non-ketamine treated			Ketamine treated			P value
	Negative control (n=8)	Solvent control (n=8)	Rimonabant control (n=8)	Ketamine treated (n=8)	CLZ treated (n=8)	Rimonabant /CLZ treated (n=8)	
Percentage of change in MT Median (Quartiles)	-6 (-9.5, -5)	-5 (-9, -2)	2.5 (-7, 6)	-14[a] (-19, -3)	-6[c] (-17, 1)	3[a, c, d] (0.8, 12)	0.008*
Active swimming time FST (m) Median (Quartiles)	3.3 (3.2- 4)	3.4 (2.9- 4)	3.8 (3.5- 4.2)	2.2 [a] (1.4- 2.7)	3 [a, c] (2.5- 3.3)	4 [c, d] (3.3 - 4.4)	0.001*
Dopamine (pg./mg) Median (Quartiles)	137 (129, 137)	161 (133, 181)	126 (92, 148)	250 [a] (201, 360)	104 [c] (76, 138)	145 [c] (134, 153)	0.001*
Serotonin (pg./mg) median (Quartiles)	63 (32, 91)	64 (55, 83)	43 (28, 55)	152 [a] (119, 186)	25 [c] (19, 47)	56 [c, d] (45, 76)	0.001*

MT=Morris water maze test; *=statistically significant; (m) =minute; pg=picogram
[a] = significantly different from negative control's = significantly different from ketamine control;
[c] = significantly different from the CLZ-treated model

DISCUSSION

Our study showed that orally administered CLZ produced a significant weight gain and relative increase in heart/body weight ratio. This comes in line with results of liu et al, who found CLZ increased the heart/body weight ratio significantly. [37] [38]

On the ECG CLZ caused tachycardia and PR interval shortening. It also significantly prolonged the corrected QT interval. Ischemic changes in ECG in terms of T wave flatting or inversion and ST segment depression were also noted. In line with the present work, Strinic et al., for example reported prolongation of QTc interval in animals treated by CLZ. [39] QTc interval Prolongation is a powerful indicator for drug ability to cause Torsade de point. [40] In the present study CLZ's cardiotoxicity was confirmed by biochemical analysis of animals' sera as well as the heart tissue which showed elevated levels of inflammatory markers. In line with these results, CK-MB, AST and LDH have shown significant increases with 25 mg/kg/d dose of CLZ [41] also CLZ caused myocarditis. [42]

Similar results were reported in rat ventricular cardiomyocytes [43] Bakhshii et al; reported that CLZ markedly raised cardiac MDA levels and myocardial lipid peroxidation. [44] When monitoring the clinical course of drug-induced cardiac toxicity, the elevated serum levels of these inflammatory markers are particularly sensitive indicators of early and late heart injury. [45]

Histopathological analysis of heart tissues showed moderate inflammation with inflammatory cells recruitment and also moderate to marked fibrosis. In previous studies, CLZ treatment induced toxic myocarditis and degenerative changes accompanied by lymphocytic infiltration in cardiac sections. [38] These changes included focal sub-endocardial fibrosis, significant interstitial edema, and perinuclear vacuolation. [25]

Rimonabant prevented the body weight gain induced by clozapine treatment, while rimonabant control animals showed significant weight loss. This was in line with the body weight lowering effect demonstrated in studies using rimonabant. It was found that rimonabant (3 or 10mg/kg) significantly reduced rat body weight when it was administered once daily for consecutive 21 days. [46] Also reported by Simon and Cota [47], Several investigations in rodents suggested that part of the long-lasting reduction in body weight was attributed to rimonabant leptin-lowering activities. [48] [49] The beneficial effects of rimonabant on metabolic regulation include reducing lipogenesis and improving insulin resistance mediated via peripheral CB1 receptors. [50] In contrast, Ettaro et al. [51] reported that the 3 mg/kg dose produced non-significant reduction in weight gain, compared to statistically significance values of the 10 mg/kg dose.

In the present study rimonabant treatment attenuated ischemic changes induced by clozapine treatment as recorded by ECG. Similar to these findings, the CB1 antagonist has been shown to be protective against heart ischemic injuries. [52, 53] Additionally, pharmacological inhibition or genetic deletion of CB1 receptors decreased the heart dysfunction, oxidative stress, inflammation, and fibrosis associated with diabetes. [54] Likely in fact Rimonabant has been demonstrated to reduce cardiac hypertrophy in chronic renal disease in mice. [52] Also rimonabant reduced cardiac remodeling and improved post ischemic cardiac functions. [55] and rimonabant significantly attenuates clozapine-induced changes in left ventricle dimensions and ejection fraction. [20]

The peripheral activities of systemically administered cannabis agonists are thought to be responsible for their cardiovascular effects. This could be explained in part by peripheral CB1R activation on autonomic nerves that innervate the heart and vascular smooth muscle. [56]

Cardiac protection offered by rimonabant could not however be explained solely on the basis of CB1 receptor blockade.

In the present study, rimonabant treatment significantly attenuated the increased levels of all inflammatory markers in animal's sera (CK, LDH and AST) and heart tissues (TNFα, IL6, MDA). Adipose tissue expansion in obesity is accompanied by macrophages infiltration which produces cytokines, such as TNFα, IL6. [57, 58] Peripheral CB_1 receptor antagonism was effective in attenuating obesity and related metabolic complications. [59] Schaich et al., reported that rimonabant markedly improved pathological hemodynamic changes and attenuated cell death, inflammation, in addition to improving the metabolic parameters. [60] Another consistent results were reported by Li et al. [35]. Also rimonabant was found to significantly reduce plasma levels of CK-MB, LDH and inflammatory cytokines induced by sepsis in rats. [61] Similar results were reported by [62]. The same effects were reported in ($CB1R^{+/+}$) and in CB1-receptor deficient ($CB1R^{-/-}$) mice, suggesting that rimonabant's anti-inflammatory effect was beyond CB1-receptor antagonism alone.

In the present study, rimonabant significantly improved all histopathological changes in heart tissues. Similar to our results, Li et al; reported that pretreatment with rimonabant, attenuated the histopathological alterations induced by clozapine treatment on Day 7 and Day 14, particularly the myocardial inflammatory score, which decreased after receiving rimonabant pretreatment for 7 days. On Day 14, it was also noted that this protective impact had resulted in a notable reduction in the fibrosis-affected areas. [20] Thus if indeed rimonabant can reduce the assayed markers, this could be one of the mechanisms it attenuates cardiac changes.

In the present study, clozapine administration in schizophrenic animals showed significant effect on memory enhancements as confirmed by Morris Water Maze Test. It has also produced a signifi-

cant reduction of brain tissue dopamine and serotonin levels induced by ketamine pretreatment. In line with our results clozapine corrected pharmacological-induced learning and memory impairment, supporting its role as an effective treatment for cognitive dysfunctions in schizophrenia. Similar results were reported by [63] and [64]

Regarding the forced swimming test, clozapine treated animals showed less evidence of depression as manifested by decreased immobility time and increased active swimming time as compared to ketamine only-treated schizophrenic animals. Similar findings were demonstrated by Todorović & Filipović and by Morais et al. [65] [66] The latter group in addition showed that the intermediate dosages of clozapine (25 mg kg) showed the most desired effects on anti-depressive behavior in FST.

In the present study, rimonabant treatment in schizophrenic animals produced significant reduction in levels of serotonin and dopamine in brain tissues induced by initial ketamine treatment, yet the average levels were higher than those of clozapine treated animals yet were very close to normal values.

In comparison to those of clozapine alone-treated rats, those given rimonabant also showed further improvement in memory and learning retention in the outcomes of the Morris maze test. Also, in forced swimming rimonabant decreased immobility time to a near control values indicating antidepressive effect of rimonabant.

Melis et al. have demonesrtated that rimonabant inhibited the rise in dopamine levels in the nucleus accumbens homogenate in a dose-dependent manner at dosages of 0.3 and 1 mg/kg i.p. [67] Similar observations were also reported by Hilário et al. [68] this could be explained by rimonabant ability to attenuate Ghrelin's effect which causes increase dopamine release in mice. [69], these reports confirm observations recoded in the current study.

All of these data support the idea that CB1 receptors and their ligands are involved in mechanisms that cause enhancement of mesolimbic dopaminergic neurons during stimulation. [70] Boggs et al. demonstrated that rimonabant did not enhance general cognitive function but improved a particular learning disability related to responsiveness to praise. [71] Wenzel and Cheer postulated that a decrease in striatal dopamine release and an increase in D1 dopamine receptors were responsible for how rimonabant affected positive reinforcement learning. [72] The hypothesis that the dopamine transporter is a main target of antipsychotic medications is supported by the fact that dopamine transporter inhibition improved the therapeutic results of antipsychotic treatments. An observation that would corroborate our theory that rimonabant improved schizophrenic animals through lowering dopamine levels. [73] Also Velikova et al. examined the effect of rimonabant on the learning and memory processes of olfactory bulbectomy rat model of depression and reported that rimonabant produced a memory enhancing effect in the operated animals and partially attenuated the memory disturbances induced by the bulbectomy. [74] previous study showed that the forced swim test's immobility time was reduced by both acute and chronic rimonabant treatment. [75] But the effect of chronic rimonabant administration on depressive like behaviour in the FST was not reported yet. [76] A study by [77], demonstrated that animals did not exhibit depressive-like behavior in the FST when given 10 mg/kg rimonabant. Additionally, rats given rimonabant for long periods did not exhibit any increase in anxiety or depressive-like behaviors. [46]

In contrast, chronic rimonabant administration induced immobility and decreased active swimming in the forced swim test. [78] They also reported that chronic administration of rimonabant had displayed depressive-like [78]; and induced anxiety-like effects. [79] Cannabis use increases positive symptoms of schizophrenia and triggers psychotic manifestations in healthy individuals. [80] In addition, researchers suggest that, the endocannabinoid system is implicated in the pathophysiology of schizophrenia. [81] [82] Likewise, Clinical evidence of the disruption of the endocannabinoid system in schizophrenia patients includes an increase in the density of CB1 cannabinoid receptors in several brain regions and a rise in animals sera and cerebrospinal fluid (CSF) levels of anandamide. [83]

CONCLUSION

The results show that CLZ is toxic to the heart and increases oxidative stress and cellular damage in the myocardium. Rimonabant is promising protective tool for the management cardio metabolic adverse effects of CLZ. With anti-inflammatory properties manifested by reduction of cytokines levels and the oxidative stress marker MDA, which

represent a risk factor contributing to both cardiac toxicity and schizophrenia pathology. Rimonabant didn't negatively affect the antipsychotic effect of clozapine; more over rimonabant possess antidepressive action manifested in FST and improving negative symptoms in schizophrenic rats.

With enhancement of learning retention and memory improvement. Likewise rimonabant effect on brain neurotransmitters involved in schizophrenia in a manner indicating that rimonabant did not worsen it.

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