A novel model possible cardiotoxicity associated with administration of Boldenone and Tramadol, alone or in combination in Wistar rats

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Research Article

Keywords: Boldenone, Tramadol, Cardiotoxicity, Troponin I, Rats

Posted Date: April 8th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1475590/v1

Abstract

A risk of cardiotoxicity has been associated with the use of anabolic-androgenic steroids (AAS) and analgesics, several deaths have been attributed to such medications. This study investigates the effects on the heart of AAS; namely, boldenone (BOLD) and opioid analgesics; namely, tramadol (TRAM) given alone or in combination, for the first time. Forty adult male rats were randomly divided into four groups; (n = 10). Normal control group, BOLD (5 mg/kg, i.m.) per week, tramadol Hcl (TRAM) (20 mg/kg, i.p.) daily, and a combination of BOLD (5 mg/kg) and TRAM (20 mg/kg), respectively for two months. Those that received BOLD and TRAM alone and in combination showed deteriorated cardiac functions, elevated serum biochemical parameters (AST, CK) and deviations in lipid profiles, elevation in oxidative and inflammatory parameters (MDA, TNF-α & IL-1β) and decrease in GSH, up-regulated cardiac troponin I as well as distorted cardiac histopathological pictures. The current study elucidated the risk of administration of these drugs for sustained periods as well as the marked detrimental effects of using these drugs in combination.

Introduction

Cardiotoxicity is a major threat that may be induced eventually after the administration of many medications. This detrimental side effect may be hidden as investigators depend mainly on cardiac electrophysiology in assessing the cardiac safety of the drugs rather than examining the effects of these drugs on the cardiac tissue. The fact that drugs might, in addition to their effect on some ion channels, also exert mitochondrial and contractile dysfunction, modification of extracellular matrix, and cardiomyocyte death, [1] has led to drug development failure as well as the withdrawal of many drugs, even after introduction to the markets over many years [2]. Oxidative stress and inflammatory biomarkers are significant in determining a wide range of cardiac dysfunction that eventually may lead to heart failure, including myocardial failure, oxidative stress, and inflammation [3]. Among these biomarkers, troponins are considered the standard for evaluation of cardiac toxicity, despite their short half-lives of a maximum of two hours [4]. There are three types of troponin protein; troponin I, troponin C and troponin T. Troponin C acts by binding to calcium to initiate the contraction of the cardiac muscle, followed by the inhibitory effect of troponin I, so both troponin C and troponin I act in conjunction to control contractions. The cardiac injury may be identified by measuring the cardiac-specific troponin T and troponin I [5]. In this study, we will shed light on some of the widely used drugs and study their safe or hazardous effects on heart function. An example of these drugs is boldenone (BOLD), an androgenic-anabolic steroid that is used in an abusive manner to increase muscle mass and strength. In previous studies, BOLD was shown to increase malondialdehyde (MDA), lactate dehydrogenase (LDH) and creatinine kinase (CK) in cardiac muscle [6]. The other drug tested is a well-known, centrally-acting analgesic, tramadol (TRAM) which exerts both opioid and non-opioid mechanisms of action. In a previous study, TRAM worsened cardiotoxicity induced by alcohol in rats, via elevating MDA and CK [7]. The harmful effects of injection of either drugs or their combination, over a two-month period, are being investigated for the first time in the current study, by determination of oxidative stress, nitrosative biomarkers and proinflammatory cytokines,

using the ELISA technique. In addition, the gene expression of cardiac troponin I in cardiac tissue will be quantified by Quantitative real-time polymerase chain reaction. Our findings will also be confirmed by histopathological examination of cardiac tissue.

Materials And Methods

Drugs, reagents, kits, and chemicals

Boldenone undecylenate 5% oily solution was obtained from Equi-gan®; Lab Tornel, Co., Mexico. Tramadol HCl was obtained from the Ministry of Justice, Egypt. Serum aspartate aminotransferase (AST) assay kit was purchased from TECO DIAGNOSTICS, USA, Cat # A559-150. Cholesterol and HDL assay kits were purchased from Abcam, Egypt, Catalog # ab65390. Triglyceride assay kit was obtained from Abcam, Egypt, Catalog # ab65336. Reduced glutathione (GSH) assay kit and lipid peroxidation (MDA) colorimetric/fluorometric assay kit were purchased from Biodiagnostic, Egypt, Catalog # GR 25 10 and Catalog # MD 25 28, respectively. Superoxide dismutase (SOD) activity and nitric oxide colorimetric assay kits were purchased from Biodiagnostic, Egypt, Catalog # SD 25 20 and Catalog # NO 25 32, respectively. Creatine kinase (CK) ELISA kit was obtained from MyBioSource, USA, Cat # MBS727734. Tumor necrosis factor-α (TNF-α) ELISA kit was purchased from China- CUSABIO, Cat # CSB E11987r. Interleukin-6 (IL-6) ELISA kit was obtained from China | R&D Systems China Co., Ltd., Quantikine® ELISA, Cat # R6000B. Cardiac troponin I: Qiagen tissue extraction kit (Qiagen, USA, Cat # 74004) and High-Capacity cDNA reverse transcription kit (Applied biosystems, USA, Cat # 4368814). Other chemicals were of the highest commercial grade available.

Experimental protocol

Animals

Forty adult male Wistar rats (150-250 g) were purchased from the animal breeding unit at the National Research Centre, Dokki, Giza, Egypt. Rats were housed in plastic cages with free access to water and food ad libitum. Room temperature maintained at 20 ± 1 °C on a 12-hour light/12-hour dark cycle. Experiments were conducted following the Ethics and Animal Care Committee of the National Research Centre and following the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

Forty adult male rats were randomly divided into four equal groups (n = 10), Group 1 normal control receiving normal saline. Group 2 was administered BOLD (5 mg/kg, i.m) per week [8]. Group 3 received TRAM (20 mg/kg, i.p.) daily [9]. Group 4 received a combination of BOLD (5 mg/kg) and TRAM (20 mg/kg). Groups 2, 3, and 4 were treated for two months.

Sample collection and preparation

At the end of the experiment, blood samples were collected from the retroorbital plexus under a low dose of ketamine anesthesia and then centrifuged at 1500 rpm for 10 minutes. Serum was separated and kept

at -20 °C to assay AST, CK, cholesterol, triglycerides (TG) and HDL. Animals were then sacrificed by cervical dislocation under a high dose of ketamine anesthesia (60 mg/kg) and the heart is excised from each animal and cut into three parts. One part was used to prepare homogenate (MPW-120 homogenizer, Med instruments, Poland) in PBS to obtain 20% homogenate, which was kept overnight at –80°C. The homogenates were centrifuged for five minutes at 5000 x g using a cooling centrifuge (Sigma and laborzentrifugen, 2k15, Germany). The supernatant was taken immediately and used for assessment of oxidative stress biomarkers (MDA, GSH, SOD, and NO) using colorimetric kits [10, 11, 12, 13], respectively. Cytokines TNF-α and IL-6 were determined using specific ELISA kits according to the manufacturer's instructions. All results are calculated per mg total protein. The second portion was kept at –80°C for quantification of cardiac troponin genes expression using real-time polymerase chain reaction (PCR). The last part was kept in formalin 10% for histopathological studies.

Detection of gene expression of cardiac troponin in cardiac tissue by Quantitative real-time polymerase chain reaction (qRT-PCR):

Total RNA was isolated using Qiagen tissue extraction kit (Qiagen, USA) according to the instructions of the manufacturer. The total RNA was used for complementary DNA (cDNA) conversion using high-capacity cDNA reverse transcription kit (Applied biosystem, USA). Moloney murine leukemia virus (MMLV) reverse transcriptase was used for the synthesis of cDNA from RNA. Human Placental Ribonuclease Inhibitor (HPRI) was used for inhibition of RNase activity. Real-time qPCR amplification and analysis were performed using an Applied Biosystem with software version 3.1 (StepOne™, USA). The reaction contained SYBR Green Master Mix (Applied Biosystems), gene-specific primer pairs which were shown in table (1) and were designed with Gene Runner Software (Hasting Software, Inc., Hasting, NY) from RNA sequences from the gene bank. All the primer sets had a calculated annealing temperature of 60°C. Amplification conditions were: 2 min at 50°C, 10 min at 95°C , and 40 cycles of denaturation for 15 s and annealing/extension at 60°C for 10 min. The relative expression of the studied genes was calculated according to Applied Bio system software using the comparative threshold cycle method. All values were normalized to the (BETA ACTIN) genes as an endogenous control (reference gene)

Histopathological study

Autopsy samples were taken from the heart in different groups and fixed in 10% formalin saline for twenty-four hours. Washing was carried out with tap water then serial dilutions of alcohol (methyl, ethyl, and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degrees in a hot air oven for twenty-four hours. Paraffin bees' wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin stain for routine as well as alizarin red and examination through the light electric microscope [14]

Statistical analysis

Data represented as mean ± SE. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Tukey-Kramer test for multiple comparisons.

Results

Effect of Two months' administration of BOLD and TRAM alone or in combination on serum AST and CK

Administration of BOLD and TRAM alone or their combination for 2 months significantly increased serum AST concentration (by 172.76%, 82.55%, and 339.15%, respectively) compared to normal control group. The group injected with a combination of BOLD and TRAM also showed significant increase in AST concentration, compared to BOLD and TRAM groups (Table 2).

Moreover, injection of BOLD and TRAM alone or combined significantly elevated CK concentration in serum by 137.73%, 55.94%, and 228.49% respectively, as compared to the normal control group. The group injected with a combination of BOLD and TRAM showed a significant increase in CK level when compared to groups injected with BOLD or TRAM (Table 2).

Effect of Two months' administration of BOLD and TRAM alone or in combination on serum lipid profile

Rats injected with BOLD showed a significant increase in serum cholesterol and TG concentrations, by 104.4% and 111.59% respectively, compared to the normal control group. On the other hand, administration of TRAM and BOLD reduced serum HDL concentration prominently, by 32.6% and 16.58% correspondingly compared to the normal control group.

Intraperitoneal injection of TRAM did not show any significant change in serum cholesterol or TG levels as compared to the normal control group. Injection of BOLD in combination with TRAM markedly elevated cholesterol and TG serum levels, by 37.2% and 199.53% respectively, compared to the normal control group. Moreover, this combination significantly raised serum cholesterol and TG, by 51.1% and 57.6% respectively, when compared to the group injected TRAM only. It was also recorded that the group injected only with BOLD showed the highest increase in both cholesterol and TG compared to all other groups (Table 2). Serum HDL concentration was significantly decreased by concurrent administration of BOLD and TRAM by 26.73% as compared to the normal control group. Likewise, this combination showed a significant reduction in HDL level when compared to TRAM group by 12.18%. Interestingly, the group injected with BOLD, only showed the greatest reduction in HDL serum levels as compared to all other groups (Table 2).

Effect of Two months' administration of BOLD and TRAM alone or in combination on oxidative stress and nitrosative biomarkers

Effect on MDA

Intraperitoneal injection of BOLD and TRAM for two months significantly increased MDA concentration in cardiac tissue by 162.36% and 146.35% respectively as compared to the normal control group showing.

Combination of BOLD and TRAM showed a significant increase in MDA concentration by 184.34% as compared to the normal control group. Moreover, this combination exhibited significant increase in MDA concentration as compared to TRAM group. On the other hand, no significant change was recorded when compared to BOLD group (Table 3).

Effect on reduced glutathione (GSH)

Administration of BOLD and TRAM for two months significantly reduced GSH concentration in cardiac tissue, as compared to normal control group, by 62.4% and 63.45% respectively. Mixture of BOLD and TRAM showed a significant decrease in GSH concentration by 79.27% compared to normal control group. Likewise, combination of BOLD and TRAM showed a significant decrease in GSH level when compared to BOLD or TRAM groups (Table 3).

Effect on superoxide dismutase (SOD)

Injection of BOLD and TRAM diminished SOD concentration in cardiac tissue, compared to normal control group by 53% and 52.71% respectively. Co-administration of BOLD and TRAM showed a significant decrease in SOD concentration by 63.23% compared to the normal control group. Likewise, this combination showed no significant change in SOD level when compared to BOLD or TRAM groups (Table 3).

Effect on nitrous oxide (NO)

Intraperitoneal injection of BOLD and TRAM for two months increased NO concentration in cardiac tissue by 111.59% and 128.37% respectively, compared to normal control group. Combination of BOLD and TRAM amplified NO concentration by 199.53% as compared to the normal control group. Conversely, this combination showed no significant change in NO level when compared to groups injected with BOLD or TRAM (Table 3).

Assessment of inflammatory cytokines (TNF-α and IL-6)

TNF-α concentration in cardiac tissue significantly improved in BOLD and TRAM groups by 188.28% and 186.21% correspondingly, compared to normal control group. Moreover, Combination of BOLD and TRAM showed a significant increase in TNF-α concentration 461.86% compared to the normal control group. Also, this combination showed a noteworthy elevation in TNF-α concentration, compared to both BOLD and TRAM groups (Table 4).

IL-6 concentration in cardiac tissue significantly increased after two month administration of BOLD and TRAM by 146.89% and 158.57% respectively, compared to normal control group.

Co-administraion of BOLD and TRAM showed a significant increase in IL-6 concentration by 277.76% compared to the normal control group. This combination of BOLD and TRAM also showed a significant increase in IL-6 concentration, as compared to both BOLD and TRAM groups (Table 4).

Histopathological study

Cardiac tissue of normal control group showed normal histological structure of the myocardial cells in bundles (Fig. 2). The group injected with BOLD for two months showed congestion in the myocardial blood vessels (Fig. 3a) associated with thickening and sclerosis in the vascular wall (Fig. 3b). The group injected with TRAM exhibited myocardial degeneration (Fig. 4a), associated with severe dilatation and congestion of the myocardial blood vessels (Fig. 4b). Hypertrophy was detected in some focal areas in the myocardial cells (Fig. 4c). There was also thickening and sclerosis in the wall of the myocardial blood vessels (Fig. 4d). The group injected with a combination of BOLD and TRAM showed apparent congestion in the myocardial blood vessels (Fig. 5).

Discussion

Since the use of AAS and analgesics, mainly opioid analgesics, has been attributed to several deaths, [15] so there is an urgent need for a thorough study for their detrimental effects on the heart.

Boldenone undecylenate is one of the AAS used to improve growth via promoting protein synthesis, muscle growth, and erythropoiesis [16]. TRAM is an opioid analgesics used for reducing pain ranging from moderate to severe, although chronic use can be implicated to several adverse effects [17].

Oxidative stress occurs due to a disturbed balance between antioxidants and free radicals, leading to potential multiorgan damage. This imbalance may occur as a result of endogenous antioxidant depletion or may be due to a low intake of antioxidants and /or an increase in the production of free radicals and other reactive species [18].

Elevated levels of reactive oxygen species (ROS) induce the peroxidation of phospholipids. As a result of this oxidative status, NO might react with superoxide radicals, producing peroxynitrite which causes damage to DNA and proteins, leading to cell death [19]. ROS can also aggravate inflammation via activation of NF-κβ (redox-sensitive transcription factor) in addition to activation of numerous inflammatory mediators [20]. The release of free radicals causes extensive injury to the myocardium, triggering an increase in membrane permeability, which result in leakage of CK and AST and increases their concentration in serum [21]. It was also reported in a previous study that cardiotoxicity was evidenced by abnormal metabolism and biosynthesis of lipids as an increase of TGL, TCH, LDL, VLDL levels and a decrease in serum HDL level [22].

cTnI and cTnT are the cardiac isoforms of troponins that play a vital role in muscle contraction. Nevertheless, in case of structural impairment of the heart, these proteins are significantly abolished intracellularly. Accordingly, cTnI and cTnT are widely used for the detection of cardiac damage [23]. cTnI has been recorded to be the most sensitive and specific isoform for early and late diagnosis of acute myocardial damage [24, 25].

BOLD and TRAM are among the most commonly abused drugs, several detrimental effects had been attributed to their continuous use. Nevertheless, no preceding researches are studying their effects upon taking them in a mixture. In the current research, we studied the effect of BOLD and TRAM, alone and in combination, on oxidative stress and release of inflammatory mediators, as well as their effect on troponin expression in cardiac tissues.

The consequences of treatment with AAS on the cardiovascular system are poorly understood. In the current research, chronic treatment with BOLD at a dose of 5 mg/kg for two months caused an oxidative stress situation in cardiac tissues as evidenced by an increased level of MDA and reduced levels of GSH and SOD. In addition to the increased level of NO and so implicated in the pathogenesis of cardiac damage. These results are in accordance with Barakat et al. [26], who reported a significant increase in MDA and NO, as well as a decrease in GSH and SOD, in a study conducted on rat liver and kidney. We attributed the reduction in SOD activity either to its activation during conversion of reactive species into an inactive metabolite, or, secondarily to the direct inhibitory effect of BOLD of SOD activity [27]. Additionally, BOLD was found to contribute to significant elevation in inflammatory mediators, TNF-α and IL-6. Similarly, in a previous study nandrolone decanoate, an anabolic androgenic steroid, showed also a significant increase in inflammatory mediators [28]. It has also been reported that induction of NF-κβ by anabolic-androgenic steroid enhanced the expression of genes encoding proinflammatory cytokines [29]. In light of information reported by [30], we considered that the increase of these cytokines may also be associated with multiorgan failure. Previous studies on experimental animals have suggested that AAS might have a direct injurious effect on the myocardium [31]. Our results also revealed that treatment with BOLD caused a myocardial injury which was evidenced by a marked increase of serum AST and CK, which suggested myocardial disorders as reported by Brancaccio et al. [32]. Our results also revealed that treatment with BOLD showed a significant increase in gene expression of cardiac troponin I in cardiac tissue. The increase of troponin I may be attributed to structural impairment of the heart [23]. Additionally, pathological findings showing congestion in the myocardial blood vessels associated with thickening and sclerosis in the vascular wall. In a previous study, the authors recorded that administration of BOLD induced many histopathological impairments in cardiac tissue marked as, prominent hypertrophy, necrosis, marked interstitial fibrosis and leukocytic infiltration [33]. BOLD also showed a deviation in lipid profiles detected by an increase in cholesterol and TG and a decrease in HDL. Our findings are in agreement with Achar et al. [34] who reported that, administration of high doses of androgenic anabolic steroids leads to detrimental effects on plasma lipid profile via increasing TG levels and decreasing HDL-C levels by 70%.

Chronic treatment with TRAM at a dose of 20 mg/kg for two months led to a significant increase in cardiac MDA and NO and but a significant suppression in GSH and SOD activities. These effects may be attributed to the oxidative stress induced by TRAM administration that caused damage of the cardiocyte membrane via lipid peroxidation [35]. Similarly, Awadalla and Salah-Eldin [36] had noticed an elevation in serum MDA and a decrease in GSH and SOD levels, after chronic treatment with TRAM. Additionally, Mohamed and Mahmoud, [17] found that TRAM showed an upregulation of iNOS and accordingly, an increase in NO levels in the rat cerebrum. Our study also showed that TRAM injection for two months

resulted in a significant elevation of cardiac TNF-α and IL-6 levels, which signposted the ability of TRAM to induce cardiovascular inflammation. These results are in accordance with Bonizzi and Karin [20], who reported cerebral inflammation in rats treated with tramadol, evidenced by a significant elevation in cerebral TNF-α and IL-6 levels.

Furthermore, death occurred as a consequence of treatment with TRAM was evidenced by myocardial damage in autopsy findings [37]. In the current study, rats treated with TRAM for two months showed a significant increase in serum AST and CK [35], as well as upregulation of cardiac troponin I gene expression, indicating a degree of cardiac damage. Injection with TRAM for two months showed no significant increase in cholesterol and TG serum concentrations, as compared to the control group. Our findings are in agreement with Ezzeldin et al. [38], who stated that administration of TRAM up to dose of 50 mg/kg does not cause any significant change in serum biochemical parameters. On the other hand, TRAM showed a significant decrease in serum HDL, which is in agreement with the finding of Ahmed and Kurkar [39], who reported that TRAM administration decreased serum concentration of total cholesterol. This may be based on the action of TRAM on lipid metabolism or lipid peroxidation [40]. Nna et al. [41] also reported that TRAM may inhibit cholesterol synthesis. Histopathological findings showed myocardial degeneration associated with severe dilatation and congestion of the myocardial blood vessels. Hypertrophy was detected in some focal area in the myocardial cells and there were also thickening and sclerosis in the wall of the myocardial blood vessels. Similar results were obtained by Faria et al. [42], who revealed that even treatment with TRAM in analgesic doses can induce cardiac toxicity in rats.

A striking and novel observation was reported in our study, i.e. that most of the measured parameters were markedly increased in groups treated with a mixture of BOLD and TRAM. Administration of AAS and opioid analgesic, signifying their dramatic effect which may lead to cardiac arrest and sudden death.

Conclusion

In summary, we have elucidated the possible underlying mechanisms of boldenone and tramadol inducing cardiotoxicty when administered for two months. The cardiotoxicity has been attributed to MDA, IL-1β, TNF-α and troponin I up-regulation as well as deterioration in lipid metabolism. The role of these drugs alone and in combination in bringing out the unfavorable histological injury in the cardiac tissue has been also verified. We have also reported that the use of these drugs in combination has the most determintal effect.

Declarations

Acknowledgment

We are grateful to Dr. Sahar Khalil, Associate Professor of Histology & Cell biology, Faculty of Medicine, Suez Canal University, for helping us to perform the histopathological study in the current investigation.

Author contribution statement

MEA and NEM conducted the experiments.

MRK performed statistical analysis.

NEM and GFA conceived and designed the research.

MRK and GFA wrote the manuscript.

Funding

No funds, grants, or other support was received.

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Code availability

Not applicable

Ethics approval

Experiments were conducted following the Ethics and Animal Care Committee of the National Research Centre and following the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

Consent to participate: Not applicable

Consent for Publication: Not applicable

Code availability: Not applicable

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Tables

Table 1: Gene-specific primer pairs (Troponin I and βeta actin)

	Primer sequence
Troponin I	Forward primer :5'-TCAAGATGGGAGATGAGGA-3
	Reverse primer: 5'-AGTTCTGCTTCTCGGATT -3
βeta actin	Forward primer 5'-TGTTTGAGACCTTCAACACC-3'
	Reverse primer 5'-CGCTCATTGCCGATAGTGAT-3'

Table (2) Effect of Two months' administration of BOLD and TRAM alone or in combination on AST, CK and lipid profile

Groups	AST U/ml	CK Pg/ml	Cholesterol mg/dl	Triglyceride mg/dl	HDL mg/dl
Normal control	47±2.07	75.8±2.31	50±1.34	40.6±0.6	37.4±0.6
BOLD 5mg/kg	128.2±5.74[ab] [172.76%]	180.2±5.6[ab] [137.73%]	102.2±2.84[ab] [104.4%]	88.6±2.01[ab] [111.59%]	25.2±0.49[ab] [-32.6%]
TRAM 20mg/kg	85.8±3.14[ab] [82.55%]	118.2±3.48[ab] [55.94%]	45.4±1.12[b] [-9.2%]	36.8±0.92[b] [128.37%]	31.2±0.37[ab] [-16.58%]
BOLD 5mg/kg+ TRAM 20mg/kg	206.4±4.07[a] [339.15%]	249±5.9[a] [228.49%]	68.6±1.12[a] [37.2%]	58±1.34[a] [199.53%]	27.4±0.24[a] [-26.73%]

Data are expressed as (mean ± SE). Statistics were done by One-way ANOVA and confirmed by Tukey's test. BOLD: Boldenone, TRAM: Tramadol, AST: Aspartate aminotransferase, CK: Creatine Kinase, HDL: High-density lipoprotein. Numerical values between parentheses are % of change from normal control.

[a] $P<0.05$: Statistically significant from normal control

[b] $P<0.05$: Statistically significant from combination

Table (3) Effect of Two months' administration of BOLD and TRAM alone or in combination on oxidative stress and nitrosative biomarkers

Groups	MDA nmol/mg protein	GSH Mmol/mg protein	SOD U/mg protein	NO nmol/mg protein
Normal control	64.3±9.49	114.63±2.58	23.47±2.59	25.63±1.47
BOLD 5mg/kg	168.7±5.29[a] [162.36%]	±43.14.9[ab] [-62.4%]	11.03±1.64[a] [-53%]	54.23±4.27[a] [111.59%]
TRAM 20mg/kg	158.4±8.05[ab] [146.35%]	±41.96.79[ab] [-63.45%]	11.1±0.71[a] [-52.71%]	58.53±13.27[a] [128.37%]
BOLD5mg/kg+TRAM20mg/kg	182.83±4.63[a] [184.34%]	±23.761.79[a] [-79.27%]	8.63±1.99[a] [-63.23%]	76.77±11.57[a] [199.53%]

Data are expressed as (mean ± SE). Statistics were done by One-way ANOVA and confirmed by Tukey's test. BOLD: Boldenone, TRAM: Tramadol, MDA: Malondialdehyde, GSH: Reduced glutathione, SOD: Superoxide dismutase, NO: Nitric oxide. Numerical values between parentheses are % of change from normal control.

[a] *P*<0.05: Statistically significant from normal control

[b] *P*<0.05: Statistically significant from combination

Table (4) Effect of Two months' administration of BOLD and TRAM alone or in combination on TNF-α and IL-6

Groups	TNF-α pg/mg protein	IL-6 pg/mg protein
Normal control	17.33±3.26	±31.432.48
BOLD 5mg/kg	49.96±14.17[ab] [188.28%]	±77.61.17[ab] [146.89%]
TRAM 20mg/kg	49.6±6.96[ab] [186.21%]	±81.276.49[ab] [158.57%]
BOLD 5mg/kg+TRAM 20mg/kg	97.37±9.97[a] [461.86%]	±118.733.75[a] [277.76%]

Data are expressed as (mean ± SE). Statistics were done by One-way ANOVA and confirmed by Tukey's test. BOLD: Boldenone, TRAM: Tramadol, TNF-α: Tumor necrosis factor-α, IL-6: Interleukin 6. Numerical

values between parentheses are % of change from normal control.

[a] *P*<0.05: Statistically significant from normal control

[b] *P*<0.05: Statistically significant from combination

Figures

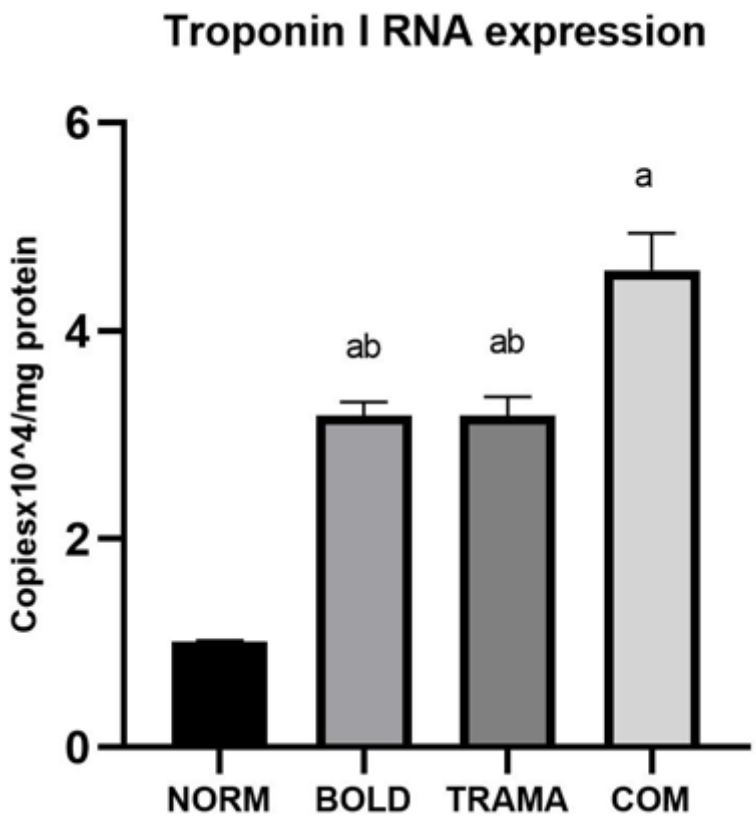

Figure 1

Effect of Two months' administration of BOLD and TRAM alone or in combination on troponin I expression in rat cardiac tissue. All data are presented as mean ± SE (n=10). P value of < 0.05 was assumed to denote statistical significance. [a]P < 0.05 Compared normal control. [b]P < 0.05 vs combination group. BOLD: Boldenone, TRAM: Tramadol, COM: Combination.

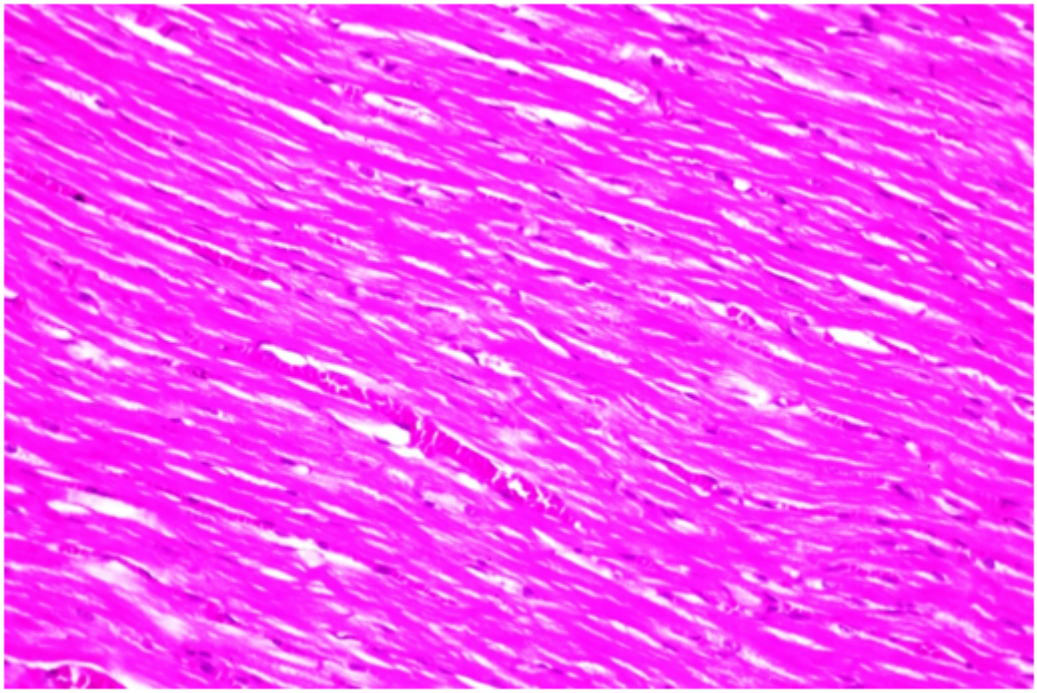

Figure 2

Cardiac tissue of normal control group showed normal histological structure of the myocardial cells in bundles. (Stain: H&E for all photomicrograph; Scale bar=100μm)

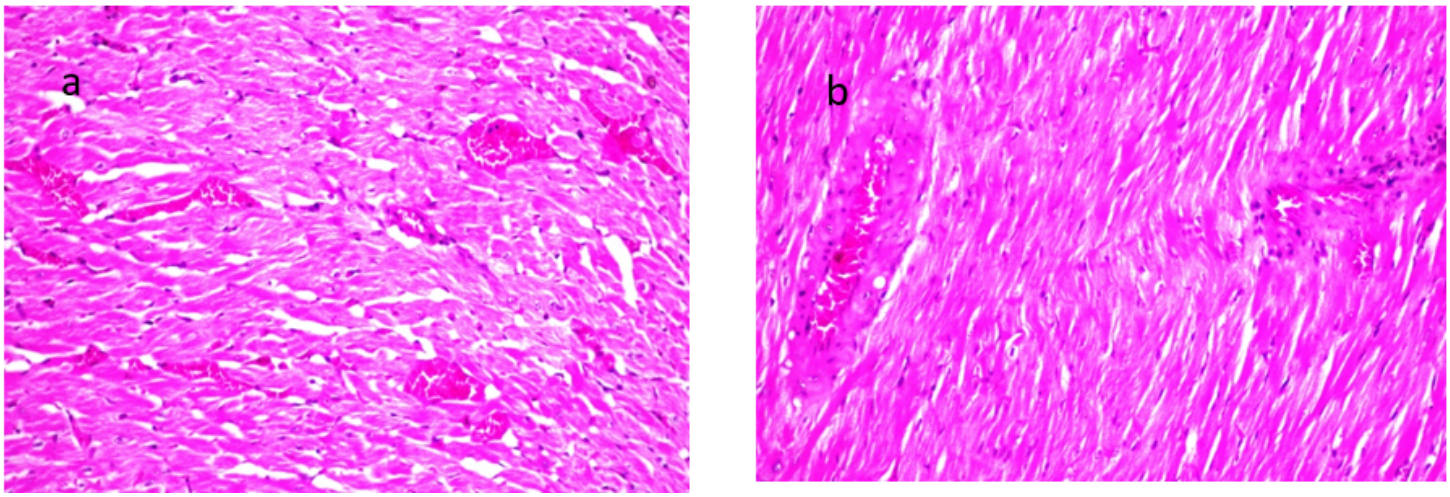

Figure 3

Cardiac tissue of group treated with BOLD (5 mg/kg) alone for two months showed congestion in the myocardial blood vessels (a) associated with thickening and sclerosis in the vascular wall (b). (Stain: H&E for all photomicrograph; Scale bar=100μm)

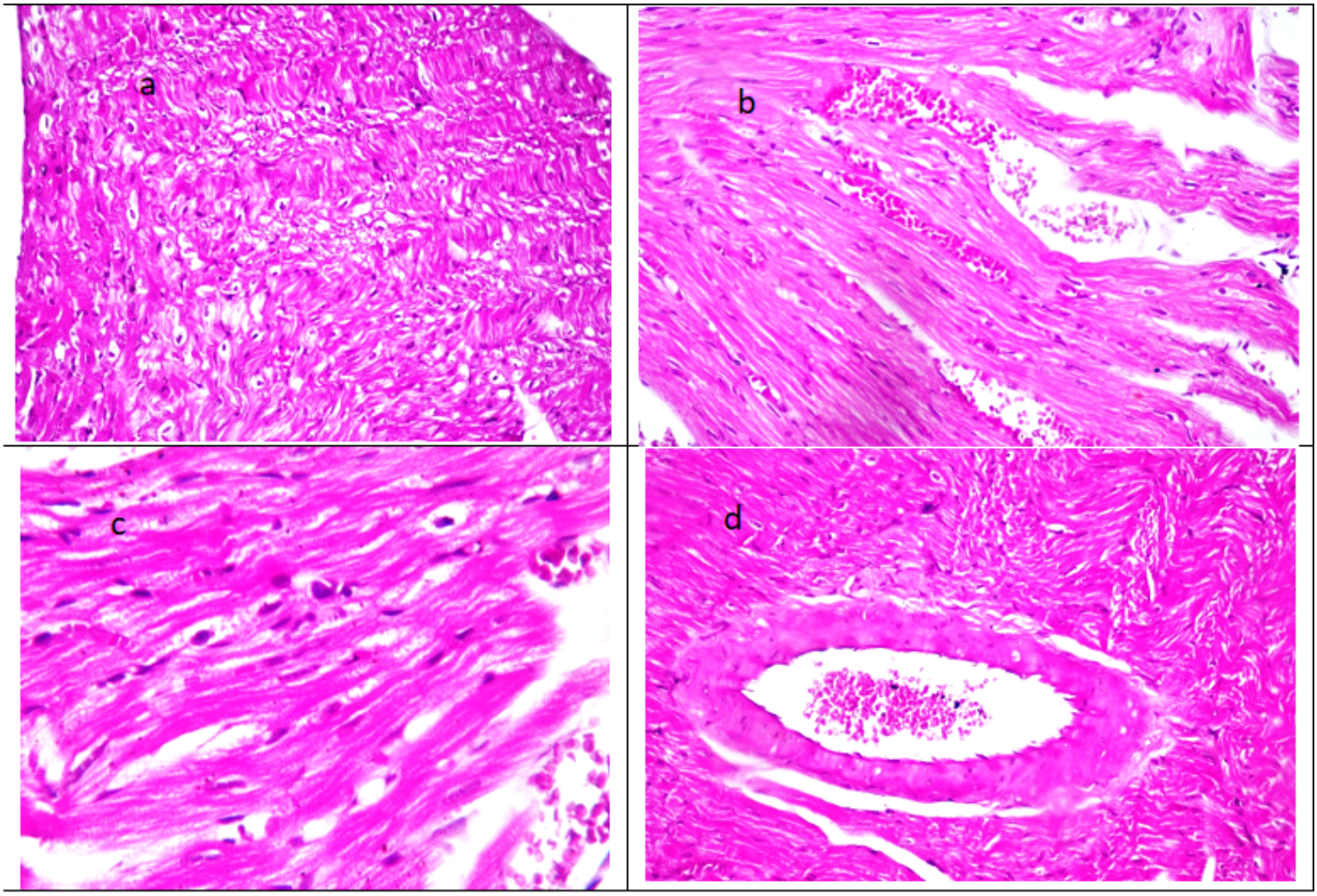

Figure 4

Cardiac tissue of group treated with TRAM (20 mg/kg) showed myocardial degeneration (a), associated with sever dilatation and congestion of the myocardial blood vessels (b). Hypertrophy was detected in some focal areas in the myocardial cells (c). There were also thickening and sclerosis in the wall of the myocardial blood vessels (d). (Stain: H&E for all photomicrograph; Scale bar=100µm)

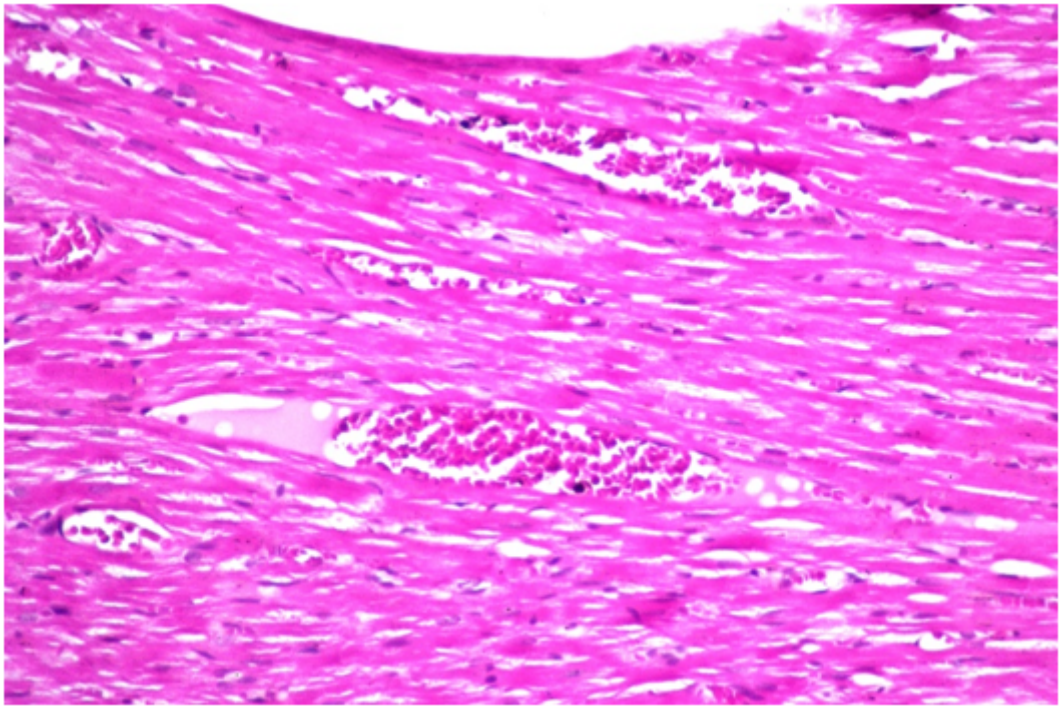

Figure 5

Cardiac tissue of group injected with combination of BOLD (5 mg/kg) and TRAM (20 mg/kg) showed apparent congestion in the myocardial blood vessels. (Stain: H&E for all photomicrograph; Scale bar=100μm)