Original Article

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Effect of lazaroid U-74389G and Sildenafil, alone or in combination, on pulmonary function during ischamia-reperfusion injury of myocardium: An exploratory study

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ABSTRACT

Background: This experimental study was designed to evaluate the effects of the pharmaceutical substances U-74389G (Lazaroid) and Sildenafil, administered separately or in combination, during anaesthesia in relation to pulmonary function, in a myocardial ischaemia-reperfusion procedure.

Methods: We sought to determine the effect of sildenafil and lazaroid U-74389G maleate, alone or in combination, on systemic and pulmonary tissue inflammation and oxidative stress in a pig model of myocardial ischaemia-reperfusion injury. A total of 56 pigs were divided into seven groups according to the use of sildenafil, lazaroid, or their combination, and the time of administration (prior to the induction of myocardial ischaemia or after reperfusion).

Results: Both sildenafil and lazaroid U-74389G maleate, administered either 10 minutes before the induction of myocardial ischaemia or immediately after the start of reperfusion, resulted in a significant effect on several markers of systemic inflammation ($p<0.05$). In addition, a significant reduction in lung tissue expression of tumour necrosis factor-alpha (TNF-a) ($p<0.05$) was observed with sildenafil, lazaroid U-74389G maleate, and their combination, when administered either before the induction of myocardial ischaemia or after the induction of reperfusion. However, no effect on tissue oxidative stress was demonstrated.

Conclusion: Sildenafil, lazaroid U-74389G maleate, and their combination led to a significant amelioration of both systemic and pulmonary tissue-specific inflammatory responses. Notably, they did not exert any significant effect against tissue oxidative stress in this model. Human mechanistic studies are required to confirm these observations.

Key Words: *Sildenafil; lazaroid U-74389G maleate; myocardial ischaemia-reperfusion; lungs injury*

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INTRODUCTION

Myocardial ischaemia and its complications, primarily myocardial infarction and ischaemic heart failure, represent a major cause of morbidity and mortality worldwide. Despite the development of reperfusion treatment strate-

gies, the functional recovery of a reperfused heart is often less successful than anticipated. This limited success is largely due to the phenomenon known as "myocardial ischaemia-reperfusion injury" (MIRI) [1]. This condition is characterised by the robust generation of reactive oxygen species (ROS), intracellular calcium overload, and the activation of inflammatory cascades, leading to enhanced cytokine release and cardiomyocyte apoptosis, ultimately resulting in the disruption of cell structure and cell death [1,2]. The adverse effects of ischaemia-reperfusion syndrome are not confined to the primary ischaemic tissue. Depending on the timing and degree of ischaemia, reperfusion can also cause significant damage to remote organs. The lungs are particularly vulnerable to injury following ischaemia-reperfusion of the bowel and/or liver, with respiratory dysfunction often being one of the first clinical symptoms that precede the manifestation of other organ failures [3-5].

Sildenafil, a phosphodiesterase type 5 (PDE5) inhibitor, prevents the breakdown of cyclic guanosine monophosphate (cGMP), thereby increasing its intracellular concentration. This elevation leads to the activation of protein kinase G (PKG), which ultimately results in smooth muscle relaxation and a reduction in cytosolic calcium concentration [6]. Numerous preclinical studies have evaluated the potential role of sildenafil and other PDE5 inhibitors in protecting against myocardial ischaemia-reperfusion injury [7]. Additionally, its beneficial effects have been suggested in human mechanistic studies [8].

Lazaroid U-74389G, a 21-aminosteroid, acts as an inhibitor of iron-mediated lipid peroxidation and has been shown in experimental studies to attenuate myocardial ischaemia-reperfusion injury [9]. This compound may also be effective against target organ damage during ischaemia-reperfusion injury, such as in the lungs or the liver [10,11]. Lazaroid primarily targets oxidative stress [12], and it has been proposed to enhance the activation of the protein kinase C (PKC) signaling pathway [13]. However, it might not exert any anti-inflammatory effects [14].

Therefore, we sought to study the effect of sildenafil and lazaroid maleate U-74389G, alone or in combination, on systemic and pulmonary inflammation and oxidative stress in a porcine model of acute myocardial ischaemia reperfusion injury, prior to myocardial ischaemia and immediately after starting the reperfusion process. This particular experimental model is original and has not been studied in the existing literature.

METHODS

All experimental procedures were assessed and approved by the Institutional Ethical Review Board, and all animals received care in accordance with institutional guidelines. The experiments were also approved by the Experimental Research Center ELPEN and the veterinary authorities of the East Attica Region, in compliance with Greek Law No. 160 (A-64, May 1991), European Union regulations, and the principles of the Helsinki Declaration. The care and use of laboratory animals followed the guidelines of the European Community Council Directive on the use of laboratory animals in experiments (License Reference Number: 7156, 05/12/2012, Veterinary Authority of Attica Region, Georgios Titis).

A total of 60 Landrace pigs, both male and female, were used for this experimental study. All animals were kept in the laboratory for three-four days prior to the conduction of study procedures, with free access to food and water, under stable temperature and lighting conditions (12-hour day and 12-hour night period cycles).

The study procedures began with the sedation of the pigs under general anaesthesia with sevoflurane, chosen for its tissue-protective effects during reperfusion.

After performing a vertical cervical incision, the common carotid artery and the internal jugular vein were catheterised. Both applied catheters were connected to a monitoring device for continuous recording of systolic, diastolic, and mean arterial pressure, central venous pressure, heart rate and rhythm, and oxygen saturation. Subsequently, a median sternotomy was performed, and the left anterior descending (LAD) artery was identified. Myocardial ischaemia was then induced through the blockade of the LAD artery (Figures 1, 2).

The animals were divided into seven treatment groups, each consisting of eight pigs, based on the use of sildenafil, lazaroid U-74389G maleate, or their combination, and the timing of administration (prior to induction of myocardial ischaemia or after reperfusion). The specific pharmaceutical treatments were as follows:

- **Group A (*Control group):*** no treatment.
- ***Group B (Lazaroid-reperfusion group)***: lazaroid U-74389G maleate administered immediately after the start of reperfusion.
- ***Group C (Lazaroid group):*** lazaroid U-74389G maleate administered 10 minutes before induction of myocardial ischaemia.
- ***Group D (Sildenafil-reperfusion group):*** sildenafil administered immediately after the start of reperfusion.
- ***Group E (Sildenafil group):*** sildenafil administered 10 minutes before induction of myocardial ischaemia.
- ***Group F (Combination-reperfusion group):*** sildenafil and lazaroid U-74389G maleate administered immediately after the start of reperfusion.
- ***Group G (Combination group):*** sildenafil and lazaroid

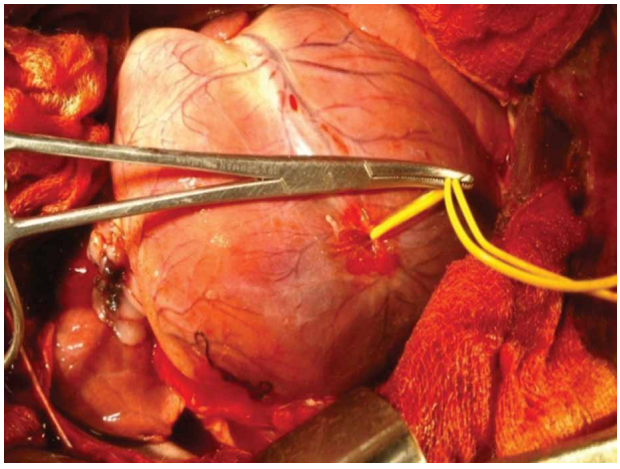

FIGURE 1. Preparation and peribronchialisation of the anterior cationic branch.

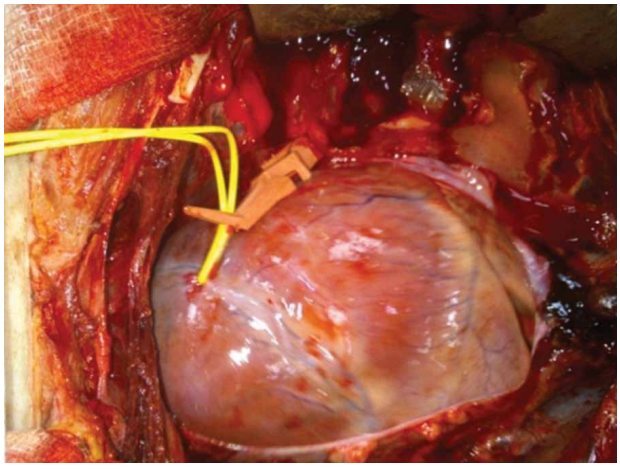

FIGURE 2. Ischaemic area of myocardium.

U-74389G maleate administered 10 minutes before induction of myocardial ischaemia.

The primary outcomes measured included markers of systemic inflammation, lung tissue expression of tumour necrosis factor-alpha (TNF-α), and tissue oxidative stress. The systemic inflammation markers included levels of interleukins (IL-1β, IL-6) and myeloperoxidase (MPO). Lung tissue samples were collected for analysis of TNF-α expression and oxidative stress markers, such as malondialdehyde (MDA) and glutathione (GSH) levels. The effectiveness of the treatments was assessed by comparing these markers between the treatment and control groups.

Study Groups and Interventions

Before assigning the animals to the study groups, we used four pigs to acquire relevant experience and identify potential errors throughout study procedures, thus overcoming the "learning curve" phenomenon. Then, 56 pigs in total were randomly divided into the following seven study groups, with each group consisting of eight animals:

- **Group A (Control Group):** This group did not receive any study drugs at any time point, serving as a control. After induction of acute myocardial ischaemia, restoration of myocardial blood supply was performed at 120 minutes.
- **Group B (Lazaroid Post-Ischaemia):** Lazaroid was given after myocardial ischaemia. At 30 minutes, acute myocardial ischaemia was induced. At the end of myocardial ischaemia, lazaroid U-74389G maleate was administered intravenously, and then restoration of LAD blocking was performed. Reperfusion lasted 120 minutes in total.
- **Group C (Lazaroid Pre-Ischaemia):** Lazaroid was given before myocardial ischaemia. After endotracheal intubation of the animal, lazaroid U-74389G maleate was administered intravenously, and after 10 minutes, myocardial ischaemia was induced for 30 minutes. Then, restoration of LAD artery blood flow was performed, for a reperfusion time of 120 minutes in total.
- **Group D (Sildenafil Post-Ischaemia):** Sildenafil was given after myocardial ischaemia. At 30 minutes, acute myocardial ischaemia was induced. At the end of myocardial ischaemia, sildenafil was administered intravenously, and then restoration of LAD blocking was performed. Reperfusion lasted 120 minutes in total.
- **Group E (Sildenafil Pre-Ischaemia):** Sildenafil was given before myocardial ischaemia. After endotracheal intubation of the animal, sildenafil was administered intravenously, and after 10 minutes, myocardial ischaemia was induced for 30 minutes. Then, restoration of LAD artery blood flow was performed, for a reperfusion time of 120 minutes in total.
- **Group F (Combination Post-Ischaemia):** The combination of sildenafil and lazaroid was given after myocardial ischaemia. At 30 minutes, acute myocardial ischaemia was induced. At the end of myocardial ischaemia, sildenafil and lazaroid U-74389G maleate were administered intravenously, and then restoration of LAD blocking was performed. Reperfusion lasted 120 minutes in total.
- **Group G (Combination Pre-Ischaemia):** The combination of sildenafil and lazaroid was given before myocardial ischaemia. After endotracheal intubation of the animal, sildenafil and lazaroid U-74389G maleate were administered intravenously, and after 10 minutes, myocardial ischaemia was induced for 30 minutes. Then, restoration of LAD artery blood flow was per-

formed, for a reperfusion time of 120 minutes in total.

Sildenafil was administered at a dose of 25 mg/kg of body weight, similar to the dose given in human adults, while lazaroid U-74389G maleate was administered at a dose of 10 mg/kg of body weight. Both drugs were given through a central venous catheter placed in the internal jugular vein.

During the study, no toxic reactions to the studied drugs were observed. There was also no death of an experimental animal.

Sampling

Blood and tissue samples were collected at the following prespecified time points: immediately after sedation with sevoflurane (0), immediately after induction of acute myocardial ischaemia with LAD artery blocking (1), 15 (2) and 30 (3) minutes after induction of acute myocardial ischaemia, at the start of reperfusion (4), and at 30 (5), 60 (6), 90 (7), and 120 minutes (8) of the reperfusion process.

The following blood markers of systemic inflammation and oxidative stress were assessed: serum interleukin-6 (IL-6), interleukin-10 (IL-10), and myeloperoxidase (MPO). Tissue expression of tumour necrosis factor-alpha (TNF-α) was assessed as a marker of acute systemic inflammation, and malondialdehyde (MDA) was assessed as a marker of oxidative stress.

Histopathological assessment of the tissue samples was performed by two independent reviewers blinded to the applied intervention.

Blood samples for the measurement of parameters of interest were initially centrifuged. Plasma samples were then stored at -80 °C. Analysis was performed with the automated analyser SIEMENS Dimension ARx (Germany, Europe). Plasma IL-6 and IL-10, ET-1, and ET-2, along with MPO, were measured using enzyme-linked immunosorbent assay (ELISA).

For the assessment of tissue expression of TNF-α and MDA, lung tissue samples were initially kept in liquid nitrogen at -80 °C. After performing the Bradford reaction, MDA was measured using BIOXYTECH® LPO Assay (OXIS International Inc.) according to the manufacturer's instructions, while TNF-α was quantified using Quantikine® Colorimetric Sandwich ELISA Kits (R&D Systems).

Statistical Analysis

Data are presented as means ± standard deviation or absolute numbers (n) with relative frequencies (%). Group differences were analysed using a two-tailed Student's t-test. For multiple comparisons, one-way or two-way ANOVA with the Bonferroni post hoc test were used. Normality was tested using the Shapiro-Wilk test. For all analyses, p values of <0.05 were considered significant.

RESULTS

i. Systemic Inflammation

Interleukin-6 (IL-6) Levels: We observed a significant decrease in IL-6 levels with the administration of sildenafil, lazaroid U-74389G maleate, and their combination immediately after reperfusion ($p < 0.05$ for the comparison of groups B, D, and F with group A). A significant decrease in IL-6 levels was also shown with the combination of sildenafil and lazaroid U-74389G maleate when administered ten minutes before the induction of myocardial ischaemia ($p = 0.041$ for the comparison between group G and group A).

Interleukin-10 (IL-10) and Myeloperoxidase (MPO) Levels: No significant differences between study groups were observed for IL-10 levels. Similarly, there were no differences in MPO levels between study groups either before the induction of myocardial ischaemia or at the start of reperfusion ($p > 0.05$ for all comparisons). Levels of MPO, IL-6 and IL-10 are presented in the Supplementary Figures 1-21.

ii. Tissue Inflammation

Tumour Necrosis Factor-alpha (TNF-α) Expression: A significant reduction in tissue expression of TNF-α was observed with sildenafil, lazaroid U-74389G maleate, and their combination when administered 10 minutes before the induction of myocardial ischaemia ($p = 0.01$ for the comparisons: group C vs. group A, group E vs. group A, and group G vs. group A).

Additionally, a significant decrease in TNF-α expression was noted with sildenafil, lazaroid U-74389G maleate, and their combination when administered immediately after the start of the reperfusion process ($p = 0.007$ for the comparison of group B vs. group A, $p = 0.011$ for the comparison of group D vs. group A, and $p = 0.005$ for the comparison of group F vs. group A). However, no significant differences were observed between the different intervention groups (groups B-G). TNF-a levels among study groups are presented in the Supplementary Figures 22-28.

iii. Tissue Oxidative Stress

Malondialdehyde (MDA) Expression: No significant effect of sildenafil, lazaroid U-74389G maleate, or their combination, administered either before the induction of myocardial ischaemia or immediately after reperfusion, was observed on tissue oxidative stress. Specifically, no differences in tissue MDA expression were observed in

groups C, E, and G compared to group A ($p = 0.092$, $p = 0.089$, and $p = 0.086$, respectively). Additionally, no differences in tissue MDA expression were noted in groups B, D, and F compared to group A ($p = 0.078$, $p = 0.081$, and $p = 0.081$, respectively). MDA levels among the study groups are presented in the Supplementary Figures 29-35.

DISCUSSION

The main results of this study indicate that sildenafil, lazaroid U-74389G maleate, and their combination significantly reduce systemic and pulmonary tissue inflammation when administered both prior to myocardial ischaemia and immediately after the myocardial reperfusion process. However, neither sildenafil nor lazaroid U-74389G maleate significantly affected tissue oxidative stress.

Mechanisms underlying the beneficial effect of sildenafil against ischaemia reperfusion injury include opening of the mitochondrial ATP-dependent potassium channels [15,16], enhanced expression and activity of endothelial and inducible nitric oxide (NO) synthase [17], upregulation of vascular endothelial growth factor (VEGF) and angiopoietin-1 (Ang-1) system leading to neovascularisation [18], PKG-dependent ERK phosphorylation [19], attenuation of oxidative stress [20], opening of the mitochondrial large-conductance calcium-sensitive potassium channels [21], and activation of the NO guanylyl cyclase (NO-GC)/ cyclic guanosine monophosphate (cGMP)-dependent signaling pathway [22]. Co-administration of sildenafil with agents such as milrinone [23] and iloprost [24] has also been shown to exert a favourable effect on myocardial ischaemia- reperfusion injury.

On the other hand, lazaroid U-74389G might have a protective effect against ischaemia reperfusion injury, mainly due to its antioxidant effects and the amelioration of the leukocyte infiltration of the ischaemic-reperfused tisue [25]. Administration of lazaroid U-74389G has been shown to exert a beneficial effect on myocardial stunning, attenuating both systolic and diastolic left ventricular dysfunction [26], while it also has a protective effect against cardiomyocytes damage and apoptosis [27].

To date, no experimental study has evaluated the effects of sildenafil and lazaroid U-74389G on systemic and pulmonary tissue inflammation in a myocardial ischaemia reperfusion injury model. In addition, there are only a few trials addressing the effect of PDE5 inhibitors on oxidative stress markers in this condition [28].

The study unveiled the pulmonary anti-inflammatory effect of either agents in a pig model of myocardial ischaemia reperfusion injury, with no observed difference between each drug alone or their combination. In addition, administration immediately after reperfusion initiation compared to early administration prior to ischaemia was not found to have a differential effect on tissue and systemic inflammation. Of note, neither sildenafil nor lazaroid U-74389G were shown to exert a significant effect against tissue-specific oxidative stress, while their combination was not efficacious against oxidative stress, as well.

Moreover, the anti-inflammatory benefits of both drugs given before myocardial ischaemia raise the hypothesis that the administration of either drug wright exerts protective anti-inflammatory effects in primary prevention.

The present study, taken together with previous experimental studies [29], extends the efficacy of bolus administration of sildenafil, lazaroid U-74389G or their combination, representing a significant step towards prompt pharmacological postconditioning on pulmonary tissue, in myocardial ischaemia reperfusion injury. However, its applicability in clinical practice and the optimal dosing regimen has to be further investigated in human mechanistic studies.

Study Novelty: This study is the first to evaluate the effects of sildenafil and lazaroid U-74389G on systemic and pulmonary tissue inflammation in a myocardial ischaemia-reperfusion injury model. Additionally, few studies have addressed the effects of PDE5 inhibitors on oxidative stress markers in this condition [28].

Potential for Primary Prevention: The anti-inflammatory benefits observed with pre-ischaemia administration of both drugs suggest potential protective effects in primary prevention.

Clinical Implications: The study results represent a significant step towards pharmacological postconditioning for pulmonary tissue in myocardial ischaemia-reperfusion injury. However, further experimental evaluation and human mechanistic studies are required to confirm these findings and determine the optimal dosing regimen for clinical practice.

Study Limitations: The absence of molecular mechanism assessments and echocardiographic evaluations before and after interventions are noted limitations that could provide additional insights.

CONCLUSIONS

Sildenafil, lazaroid U-74389G maleate, and their combination significantly ameliorate both systemic and pulmonary tissue-specific inflammatory responses in a porcine model of myocardial acute ischaemia-reperfusion injury. These findings add significant knowledge with potential direct clinical applicability. Future human mechanistic studies are needed to assess the applicability of these results in clinical practice.

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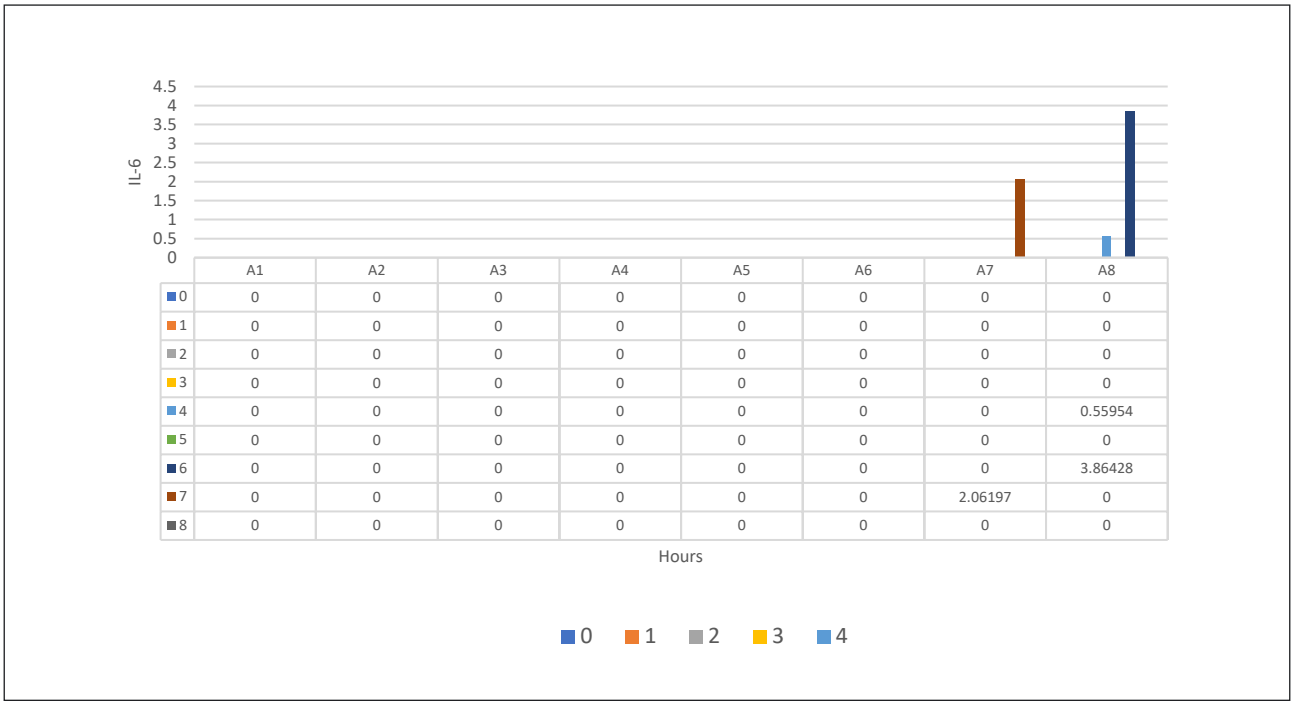
Appendix



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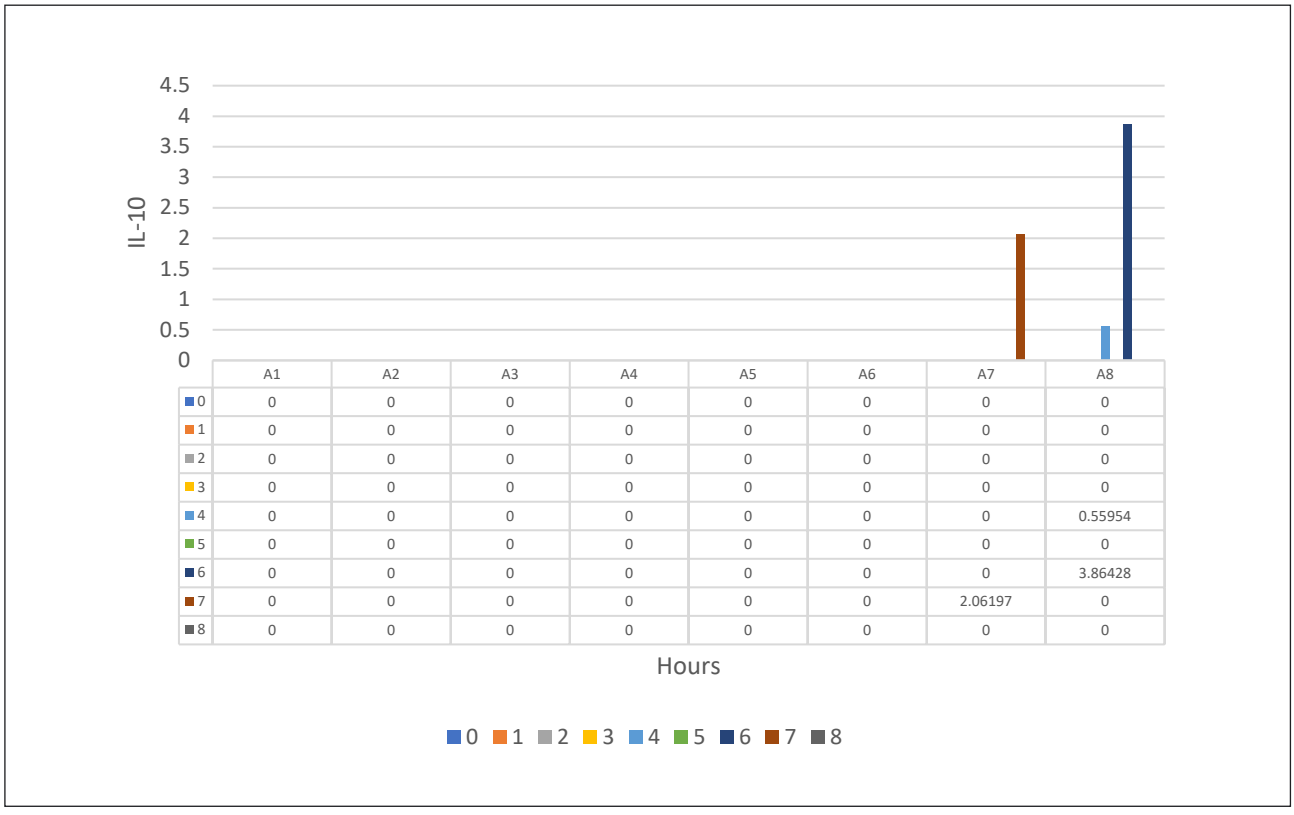
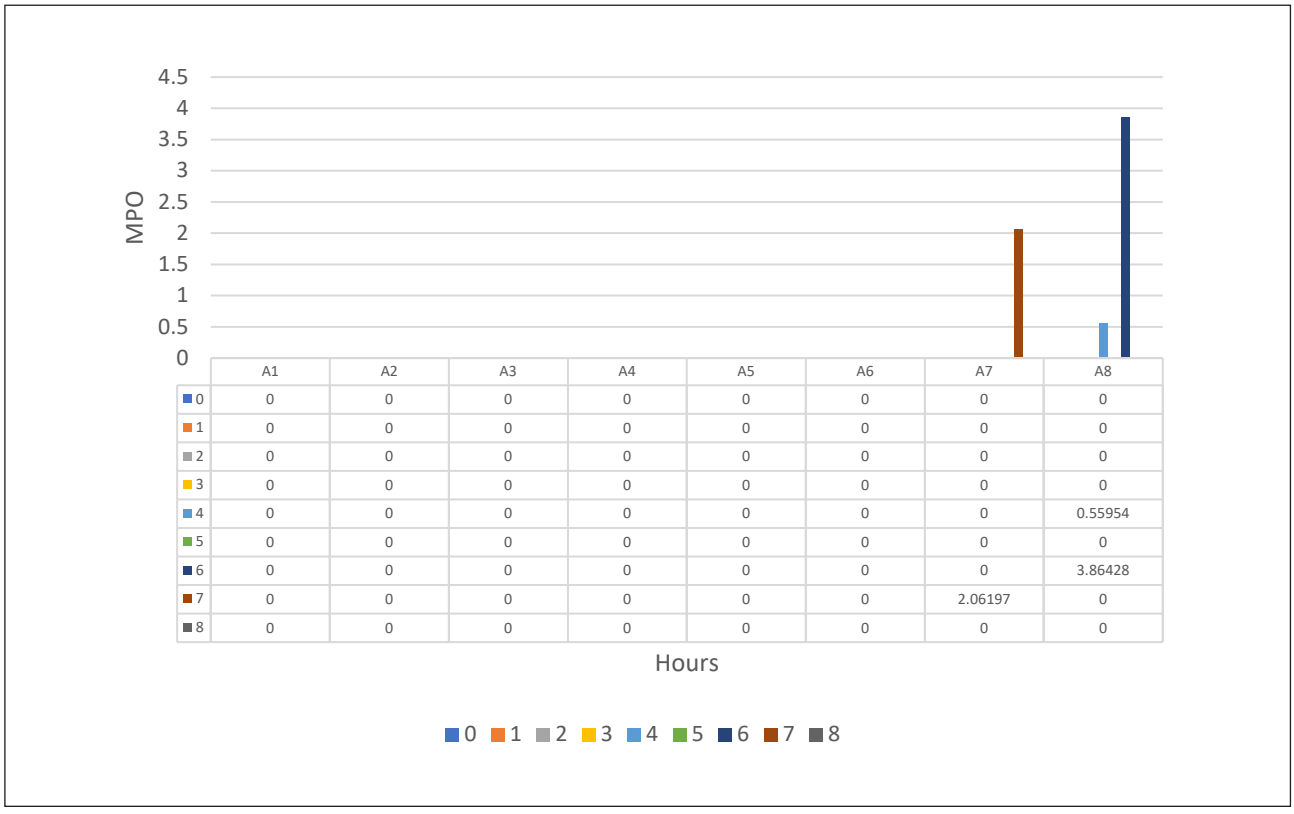
7	0	0	0	0	0	0	2.06197	0
8	0	0	0	0	0	0	0	0

SUPPLEMENTARY FIGURE 1. Group A: IL-6levels at the designated times of the study.

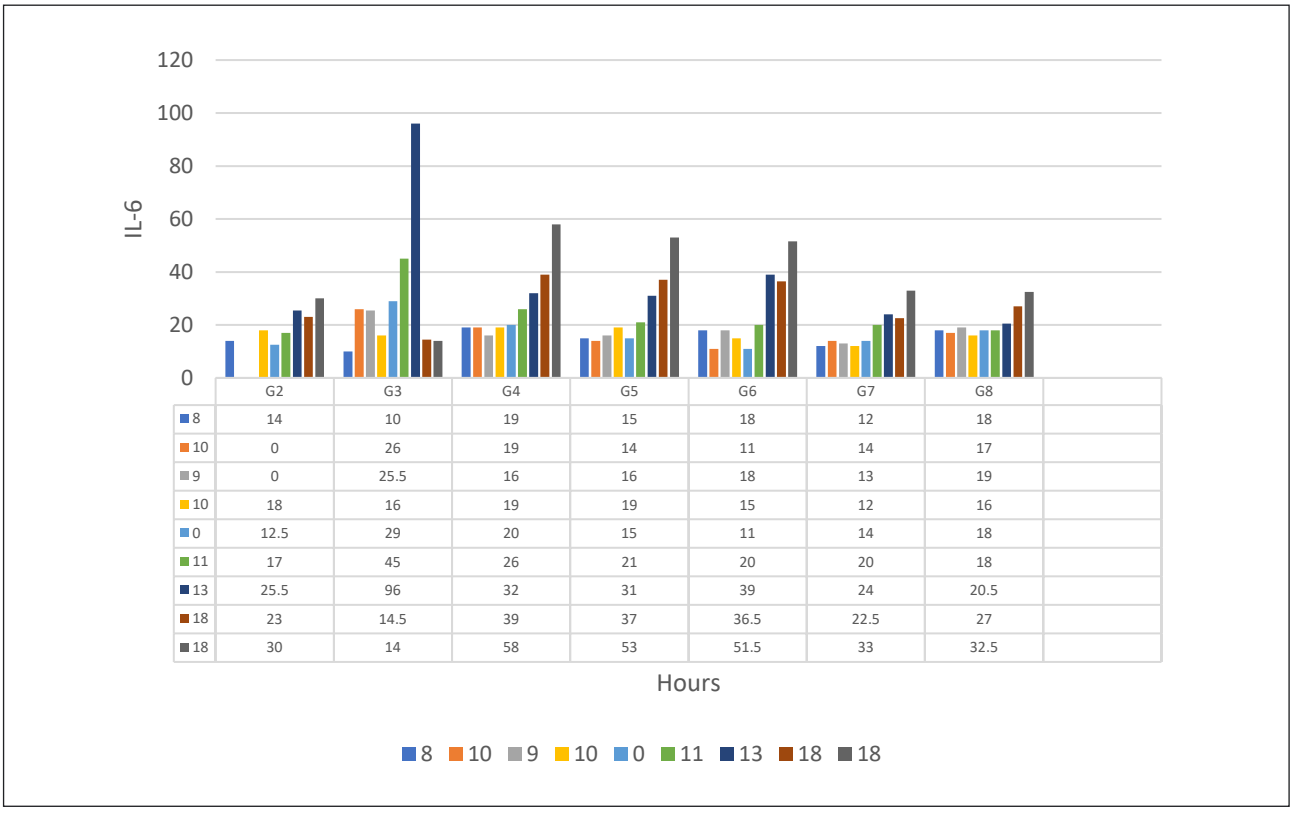


	A1	A2	A3	A4	A5	A6	A7	A8
0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0.55954
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	3.86428
7	0	0	0	0	0	0	2.06197	0
8	0	0	0	0	0	0	0	0

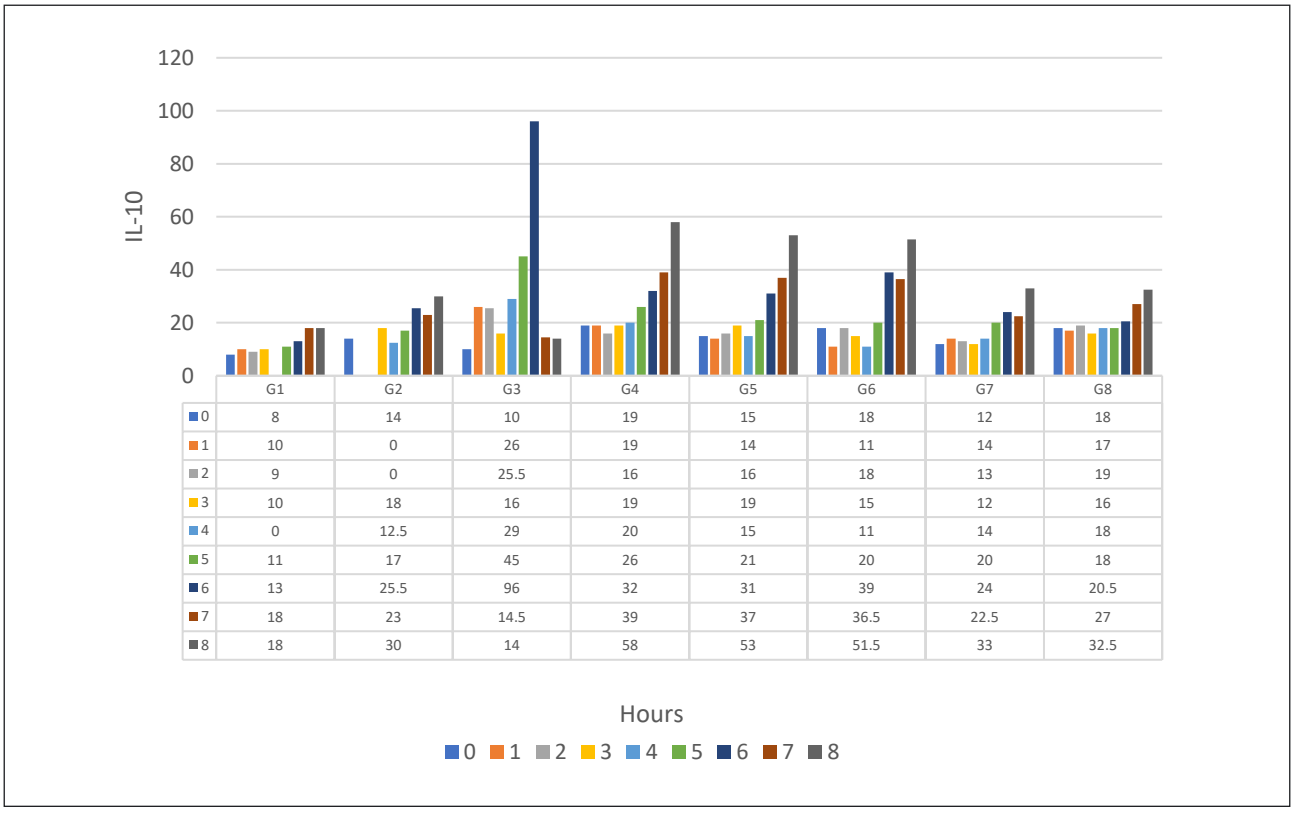
SUPPLEMENTARY FIGURE 2. Group A: IL-10 levels at the designated times of the study.



SUPPLEMENTARY FIGURE 3. Group A: MPO levels at the designated times of the study.

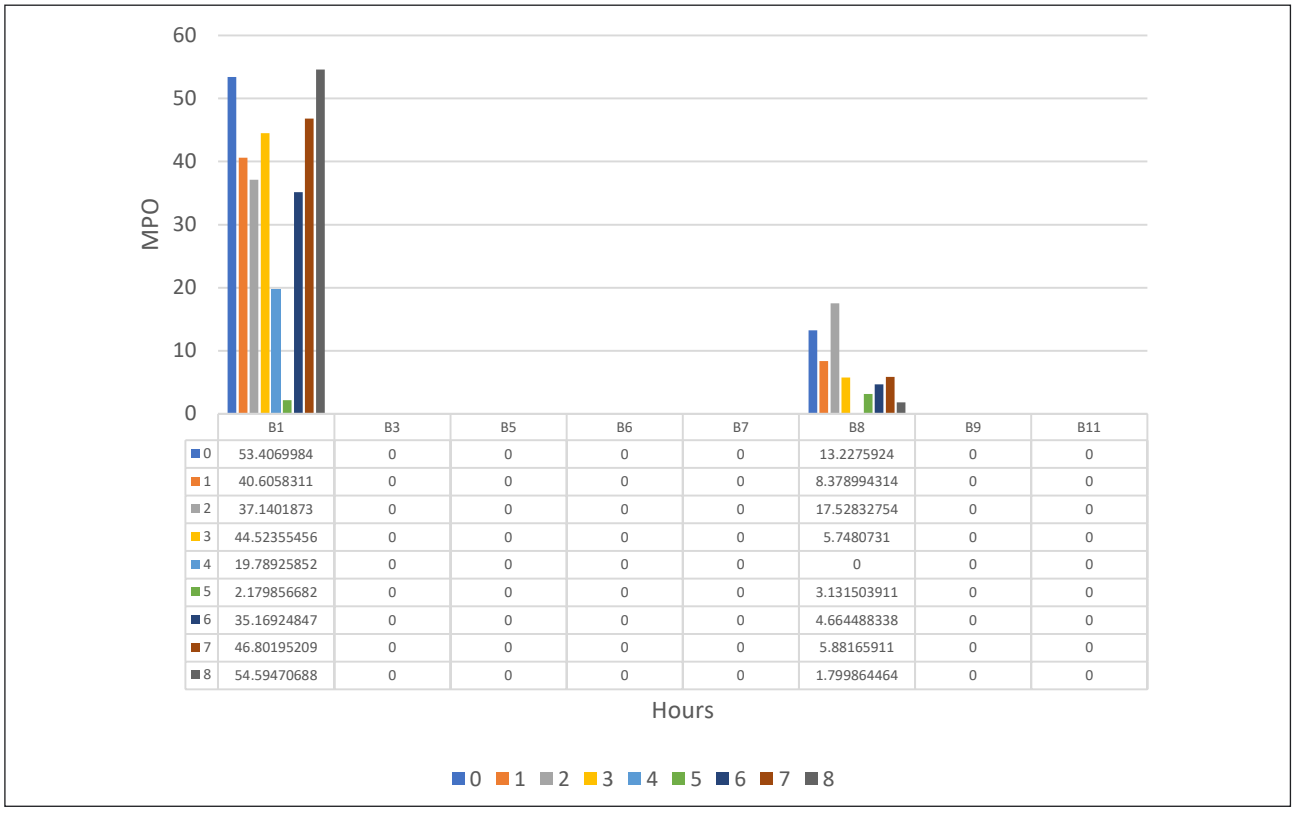


SUPPLEMENTARY FIGURE 4. Group B: IL-6 levels at the designated times of the study.



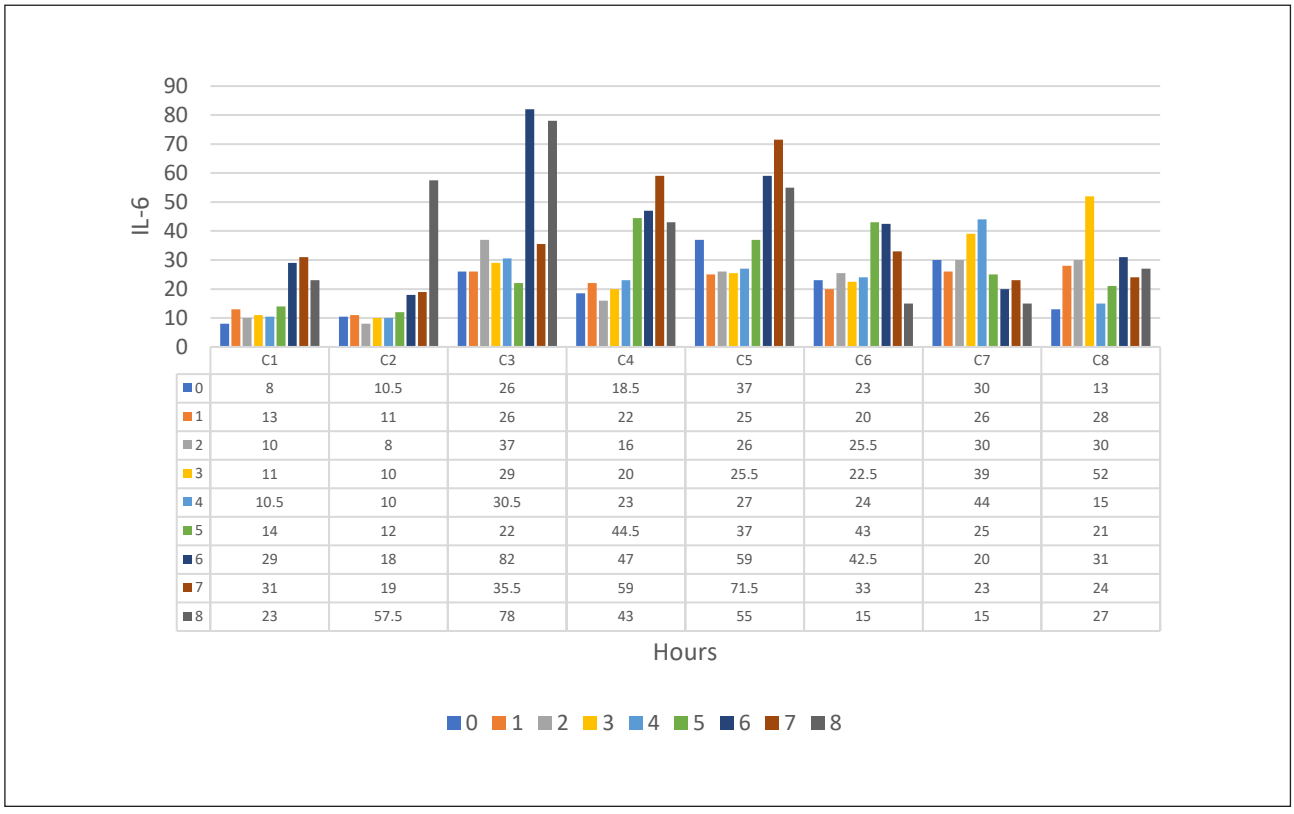
	G1	G2	G3	G4	G5	G6	G7	G8
0	8	14	10	19	15	18	12	18
1	10	0	26	19	14	11	14	17
2	9	0	25.5	16	16	18	13	19
3	10	18	16	19	19	15	12	16
4	0	12.5	29	20	15	11	14	18
5	11	17	45	26	21	20	20	18
6	13	25.5	96	32	31	39	24	20.5
7	18	23	14.5	39	37	36.5	22.5	27
8	18	30	14	58	53	51.5	33	32.5

SUPPLEMENTARY FIGURE 5. Group B: IL-10 levels at thedesignated times of the study.



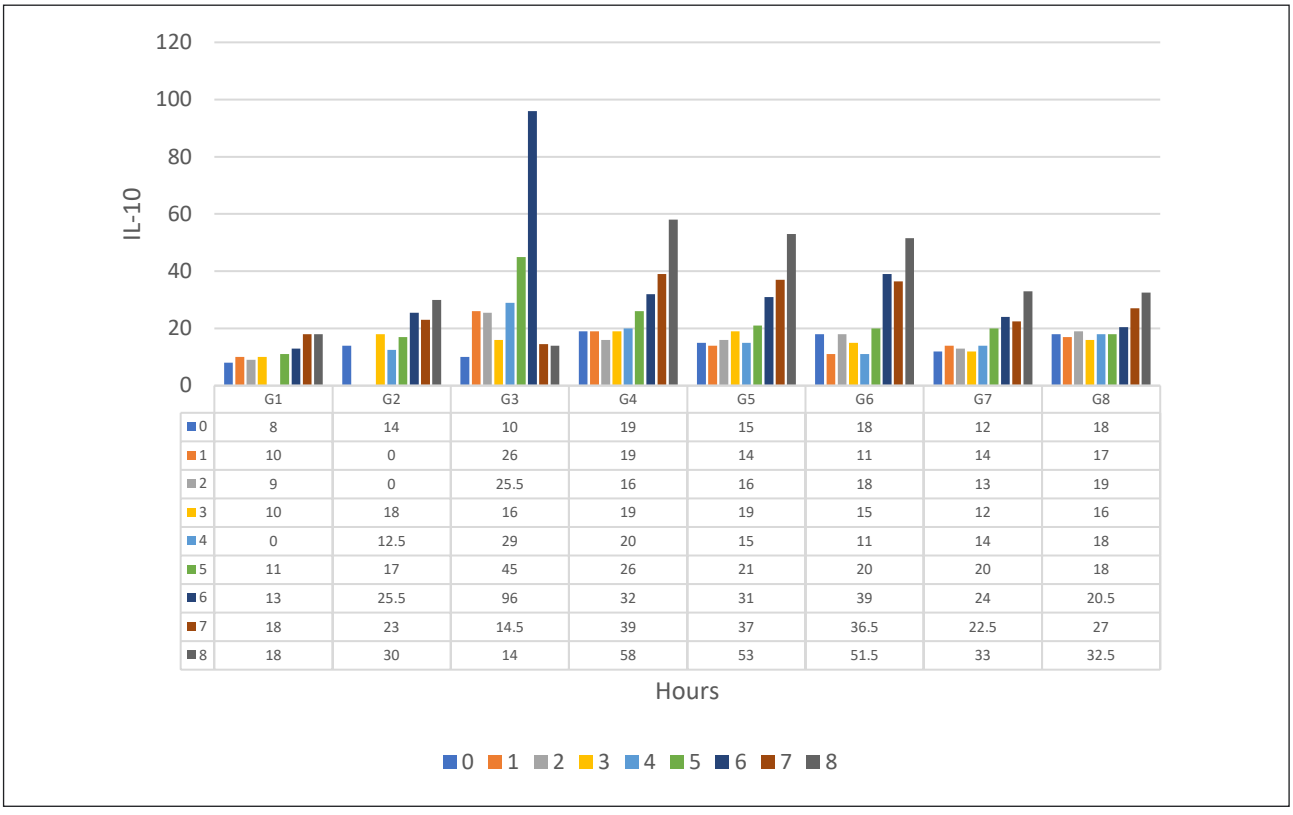
	B1	B3	B5	B6	B7	B8	B9	B11
0	53.4069984	0	0	0	0	13.2275924	0	0
1	40.6058311	0	0	0	0	8.378994314	0	0
2	37.1401873	0	0	0	0	17.52832754	0	0
3	44.52355456	0	0	0	0	5.7480731	0	0
4	19.78925852	0	0	0	0	0	0	0
5	2.179856682	0	0	0	0	3.131503911	0	0
6	35.16924847	0	0	0	0	4.664488338	0	0
7	46.80195209	0	0	0	0	5.88165911	0	0
8	54.59470688	0	0	0	0	1.799864464	0	0

SUPPLEMENTARY FIGURE 6. Group B: MPO levels at the designated times of the study.



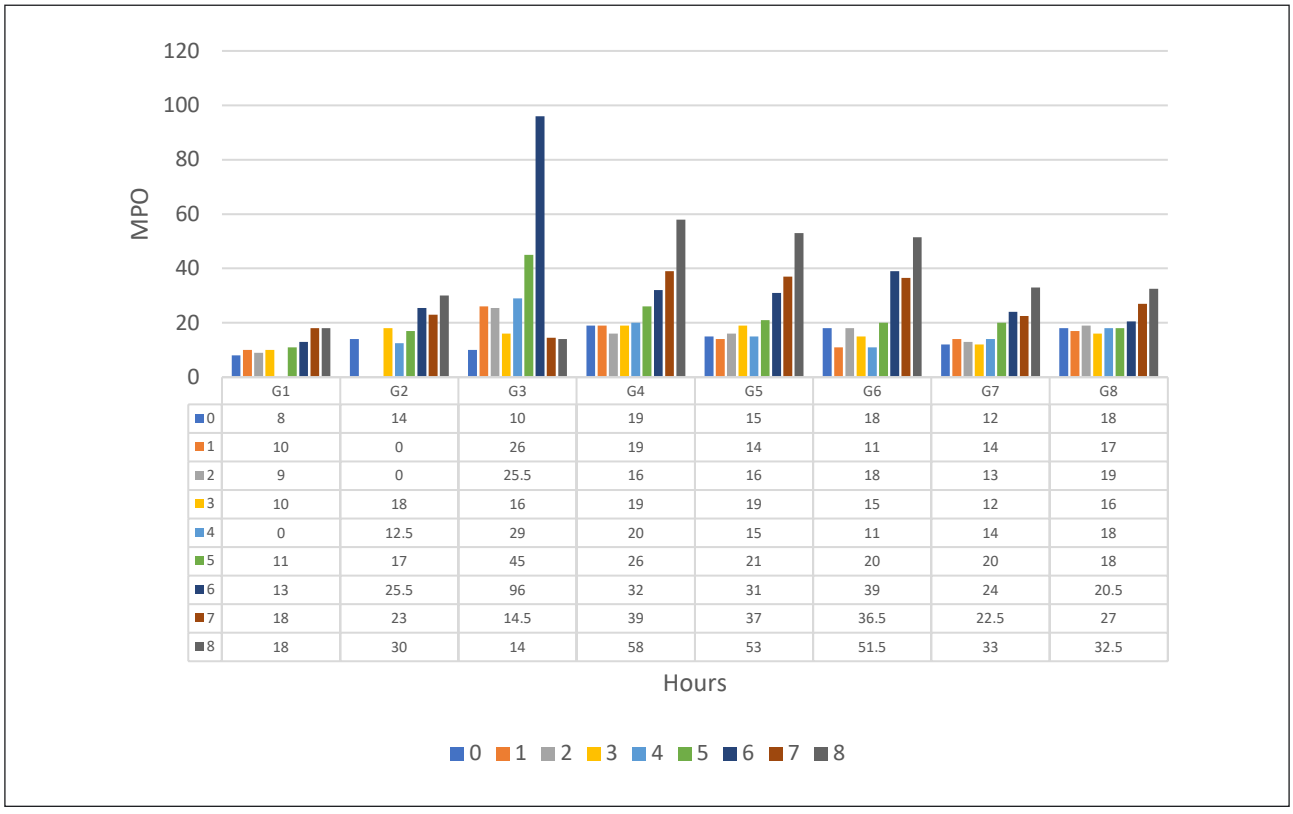
	C1	C2	C3	C4	C5	C6	C7	C8
0	8	10.5	26	18.5	37	23	30	13
1	13	11	26	22	25	20	26	28
2	10	8	37	16	26	25.5	30	30
3	11	10	29	20	25.5	22.5	39	52
4	10.5	10	30.5	23	27	24	44	15
5	14	12	22	44.5	37	43	25	21
6	29	18	82	47	59	42.5	20	31
7	31	19	35.5	59	71.5	33	23	24
8	23	57.5	78	43	55	15	15	27

SUPPLEMENTARY FIGURE 7. Group C: IL-6 levels at the designated times of the study.



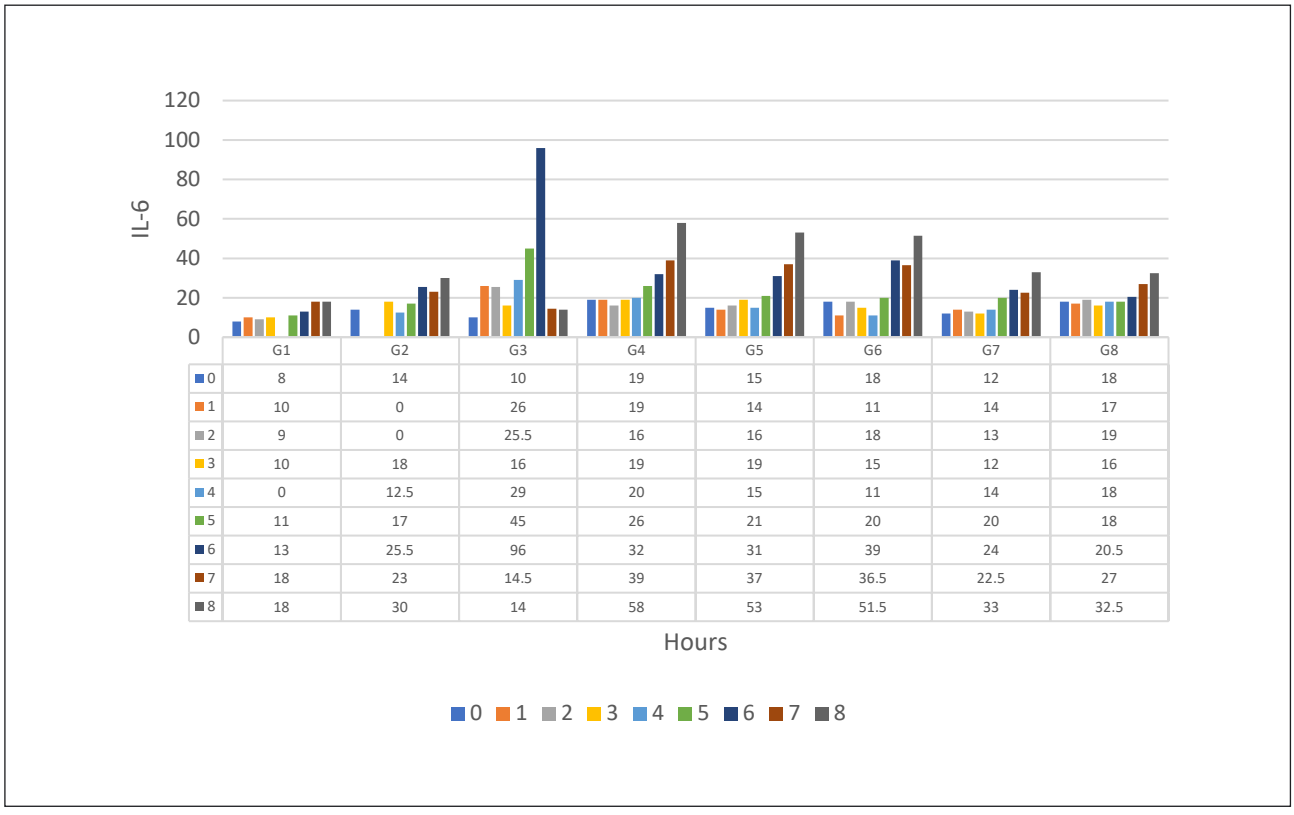
	G1	G2	G3	G4	G5	G6	G7	G8
0	8	14	10	19	15	18	12	18
1	10	0	26	19	14	11	14	17
2	9	0	25.5	16	16	18	13	19
3	10	18	16	19	19	15	12	16
4	0	12.5	29	20	15	11	14	18
5	11	17	45	26	21	20	20	18
6	13	25.5	96	32	31	39	24	20.5
7	18	23	14.5	39	37	36.5	22.5	27
8	18	30	14	58	53	51.5	33	32.5

SUPPLEMENTARY FIGURE 8. Group C: IL-10 levels at the designated times of the study.



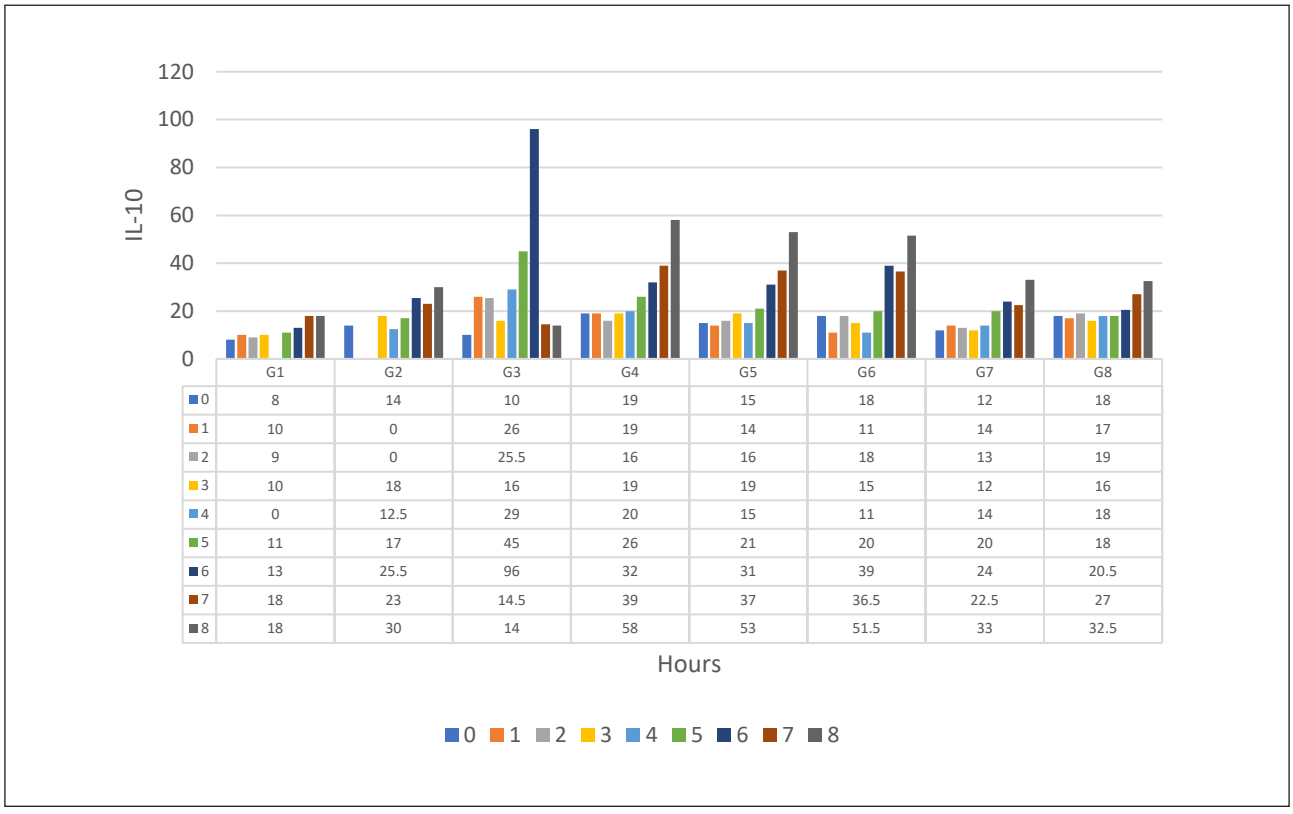
	G1	G2	G3	G4	G5	G6	G7	G8
0	8	14	10	19	15	18	12	18
1	10	0	26	19	14	11	14	17
2	9	0	25.5	16	16	18	13	19
3	10	18	16	19	19	15	12	16
4	0	12.5	29	20	15	11	14	18
5	11	17	45	26	21	20	20	18
6	13	25.5	96	32	31	39	24	20.5
7	18	23	14.5	39	37	36.5	22.5	27
8	18	30	14	58	53	51.5	33	32.5

SUPPLEMENTARY FIGURE 9. Group C: MPO levels at the designated times of the study.



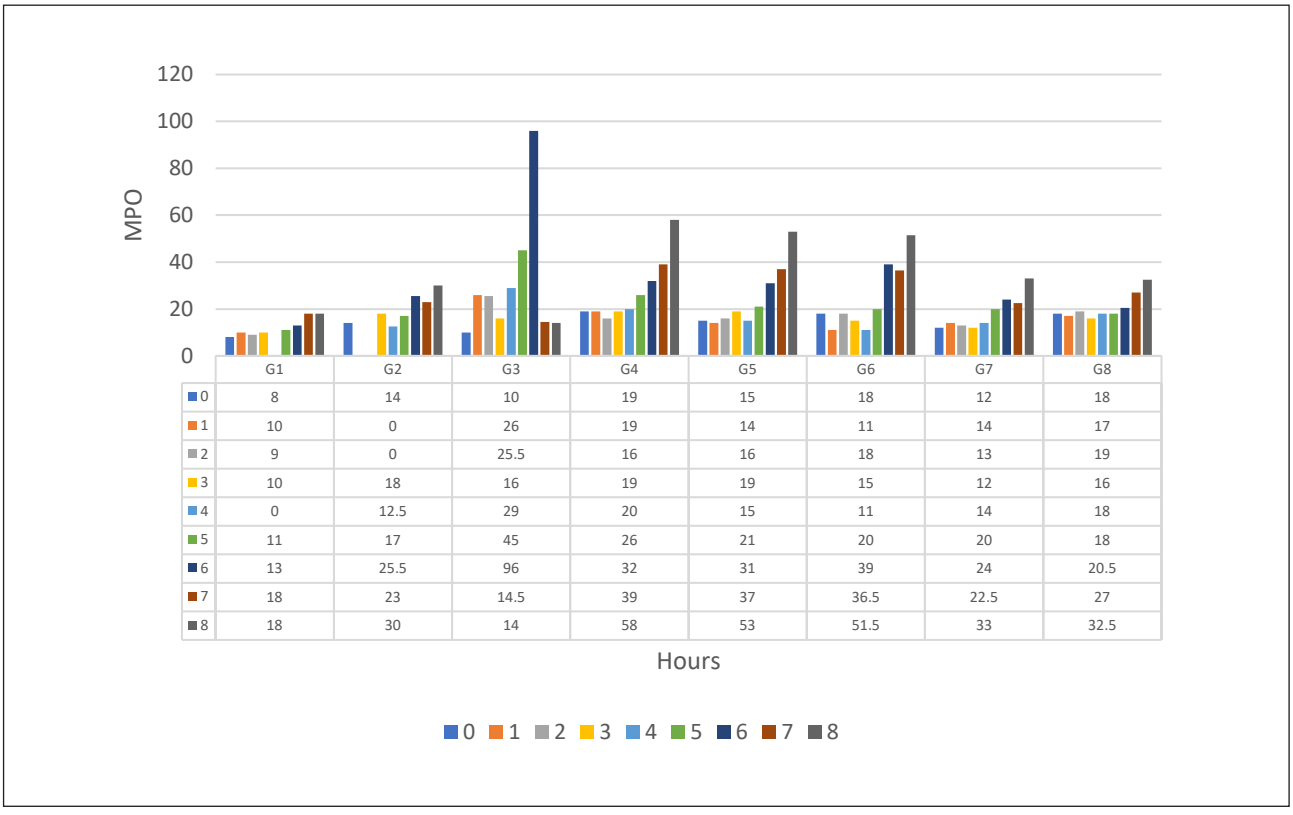
	G1	G2	G3	G4	G5	G6	G7	G8
0	8	14	10	19	15	18	12	18
1	10	0	26	19	14	11	14	17
2	9	0	25.5	16	16	18	13	19
3	10	18	16	19	19	15	12	16
4	0	12.5	29	20	15	11	14	18
5	11	17	45	26	21	20	20	18
6	13	25.5	96	32	31	39	24	20.5
7	18	23	14.5	39	37	36.5	22.5	27
8	18	30	14	58	53	51.5	33	32.5

SUPPLEMENTARY FIGURE 10. Group D: IL-6 levels at the designated times of the study.



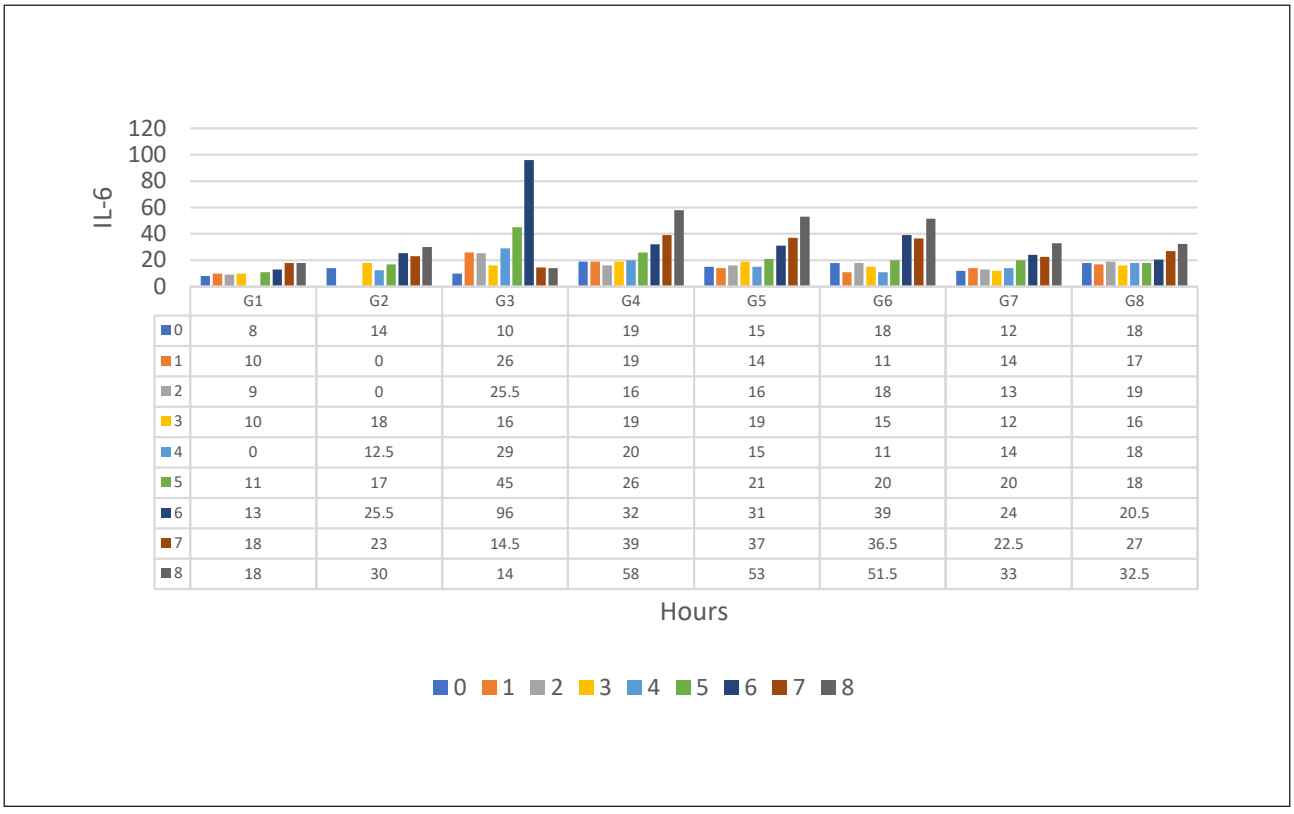
	G1	G2	G3	G4	G5	G6	G7	G8
0	8	14	10	19	15	18	12	18
1	10	0	26	19	14	11	14	17
2	9	0	25.5	16	16	18	13	19
3	10	18	16	19	19	15	12	16
4	0	12.5	29	20	15	11	14	18
5	11	17	45	26	21	20	20	18
6	13	25.5	96	32	31	39	24	20.5
7	18	23	14.5	39	37	36.5	22.5	27
8	18	30	14	58	53	51.5	33	32.5

SUPPLEMENTARY FIGURE 11. Group D: IL-10 levels at the designated times of the study.



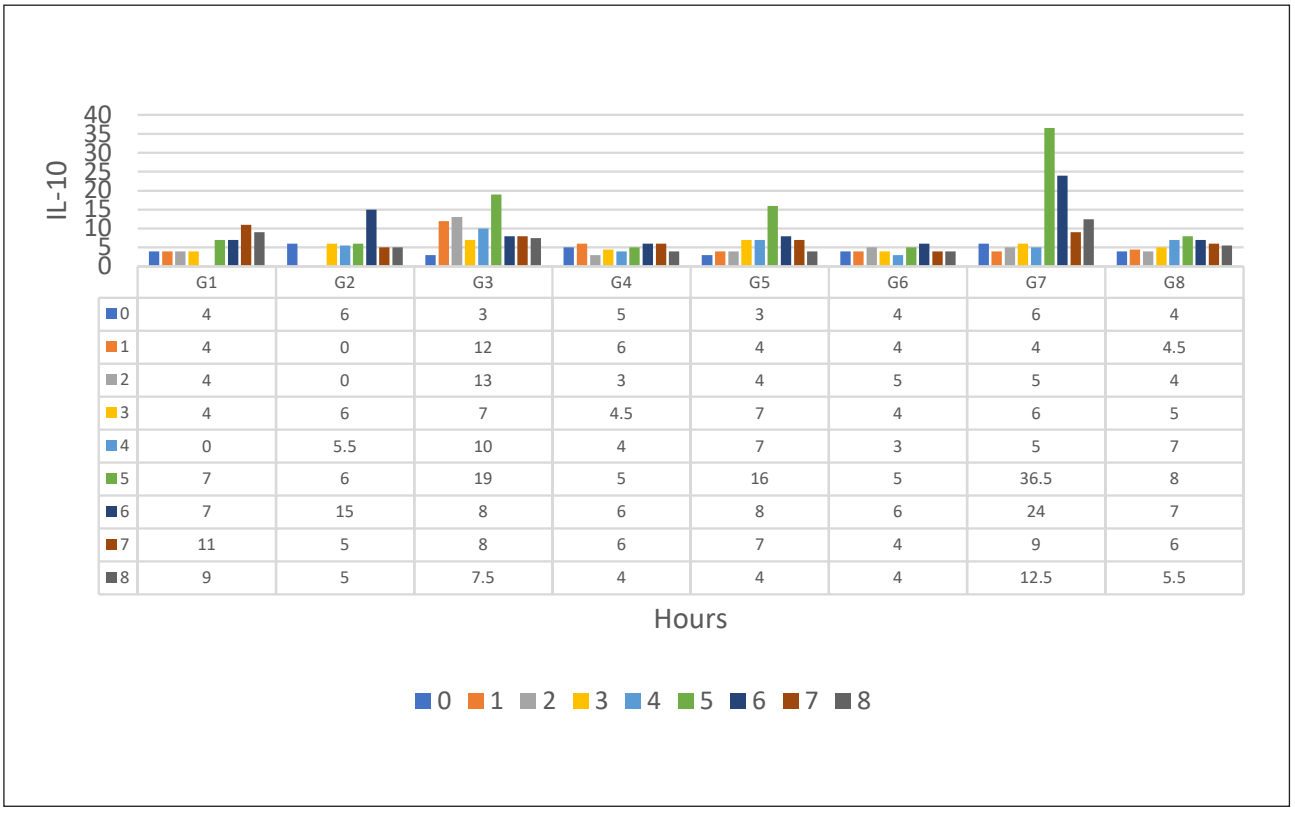
	G1	G2	G3	G4	G5	G6	G7	G8
0	8	14	10	19	15	18	12	18
1	10	0	26	19	14	11	14	17
2	9	0	25.5	16	16	18	13	19
3	10	18	16	19	19	15	12	16
4	0	12.5	29	20	15	11	14	18
5	11	17	45	26	21	20	20	18
6	13	25.5	96	32	31	39	24	20.5
7	18	23	14.5	39	37	36.5	22.5	27
8	18	30	14	58	53	51.5	33	32.5

SUPPLEMENTARY FIGURE 12. Group D: MPO levels at the designated times of the study.



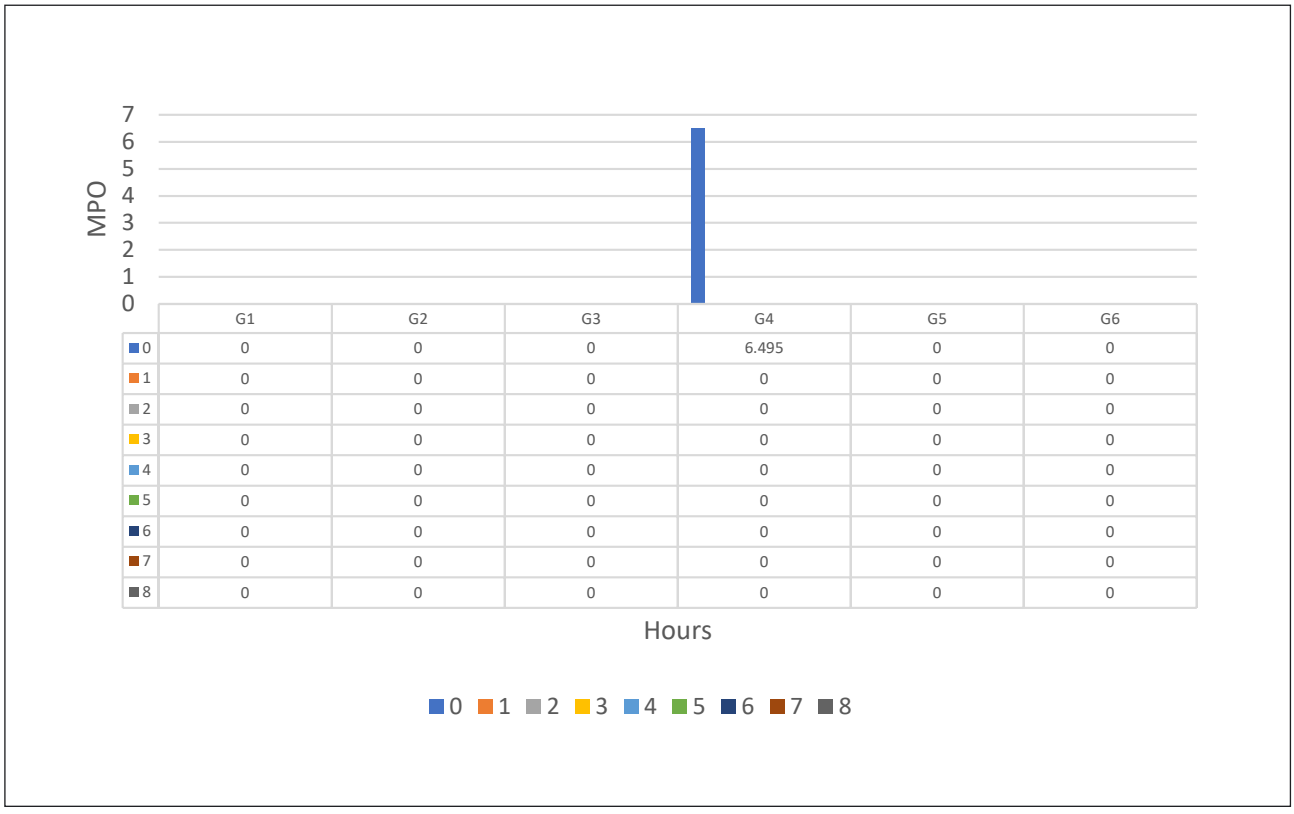
	G1	G2	G3	G4	G5	G6	G7	G8
0	8	14	10	19	15	18	12	18
1	10	0	26	19	14	11	14	17
2	9	0	25.5	16	16	18	13	19
3	10	18	16	19	19	15	12	16
4	0	12.5	29	20	15	11	14	18
5	11	17	45	26	21	20	20	18
6	13	25.5	96	32	31	39	24	20.5
7	18	23	14.5	39	37	36.5	22.5	27
8	18	30	14	58	53	51.5	33	32.5

SUPPLEMENTARY FIGURE 13. Group E: IL-6 levels at the designated times of the study.



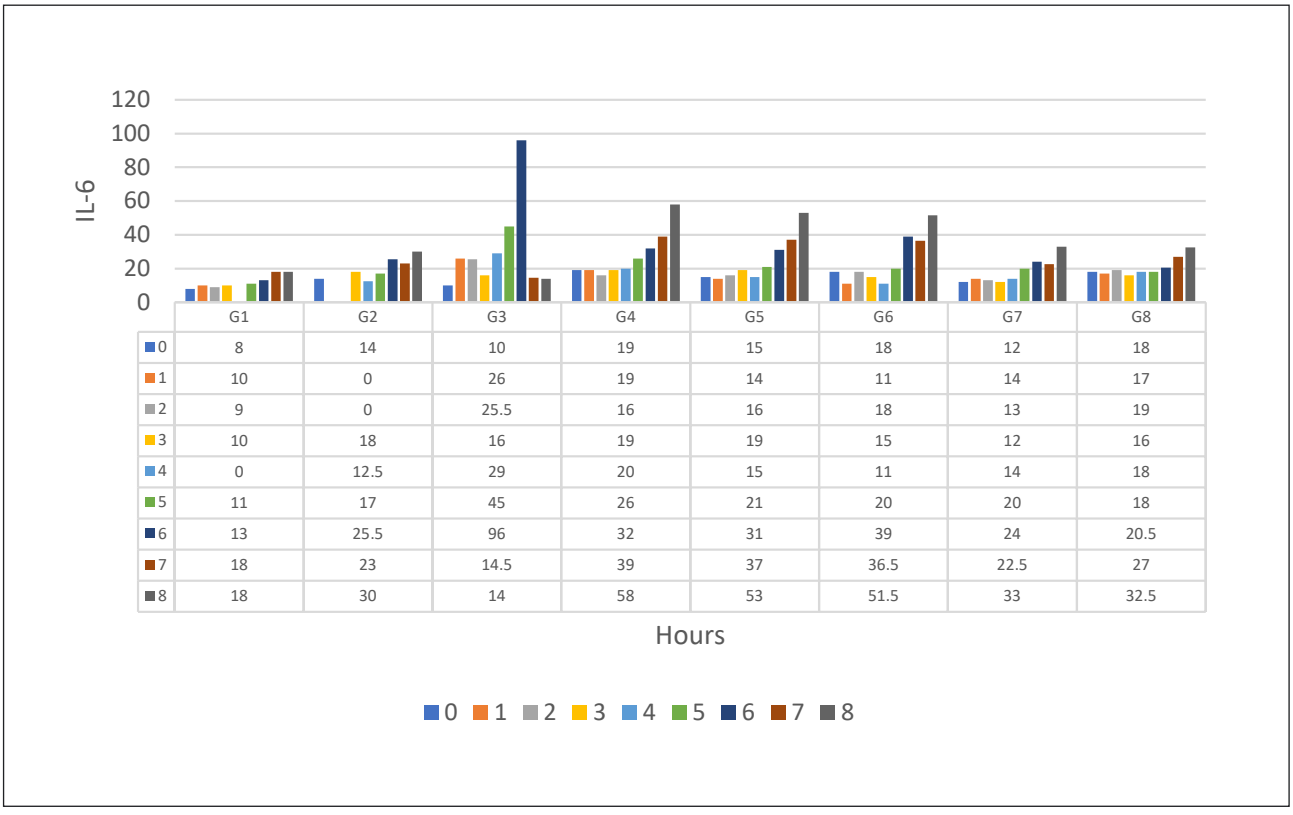
	G1	G2	G3	G4	G5	G6	G7	G8
0	4	6	3	5	3	4	6	4
1	4	0	12	6	4	4	4	4.5
2	4	0	13	3	4	5	5	4
3	4	6	7	4.5	7	4	6	5
4	0	5.5	10	4	7	3	5	7
5	7	6	19	5	16	5	36.5	8
6	7	15	8	6	8	6	24	7
7	11	5	8	6	7	4	9	6
8	9	5	7.5	4	4	4	12.5	5.5

SUPPLEMENTARY FIGURE 14. Group E: IL-10 levels at the designated times of the study.



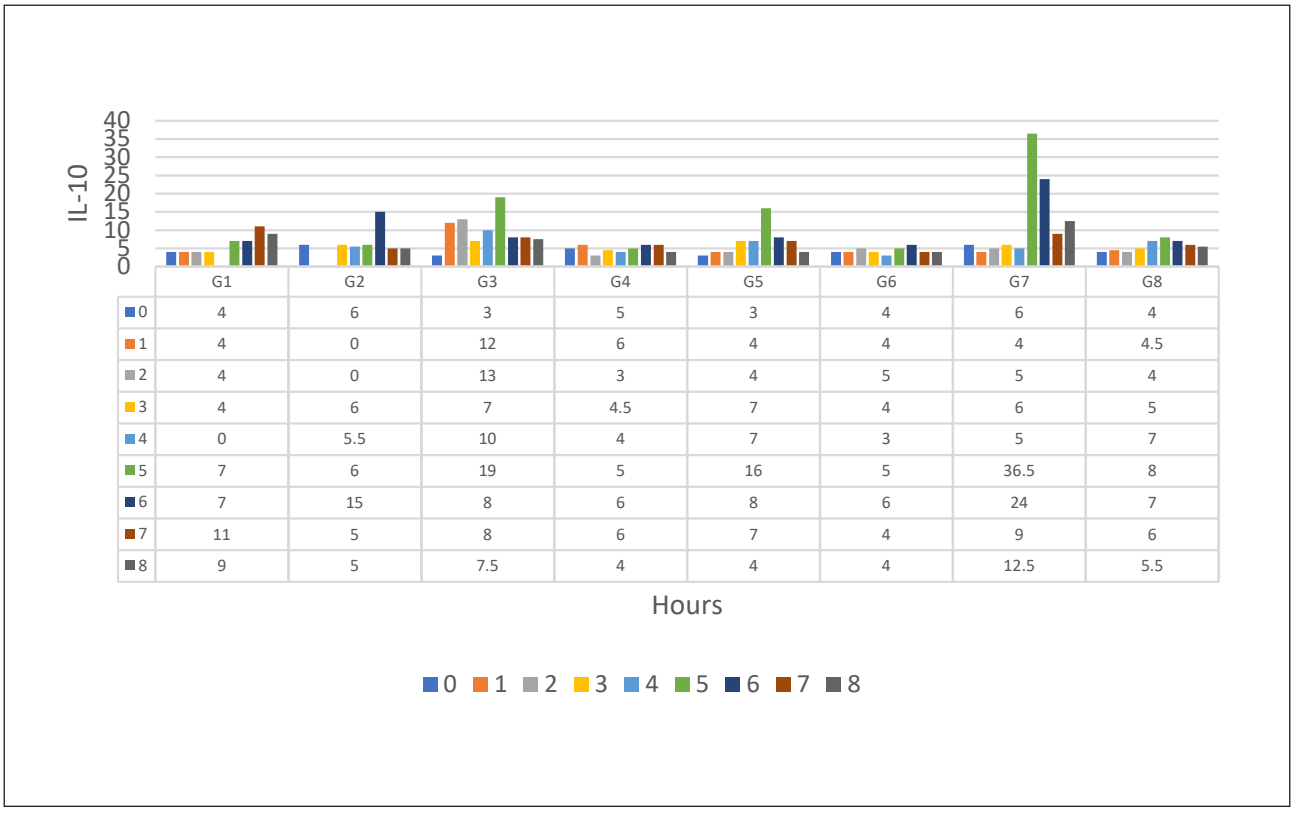
	G1	G2	G3	G4	G5	G6
0	0	0	0	6.495	0	0
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0

SUPPLEMENTARY FIGURE 15. Group E: MPO levels at the designated times of the study.



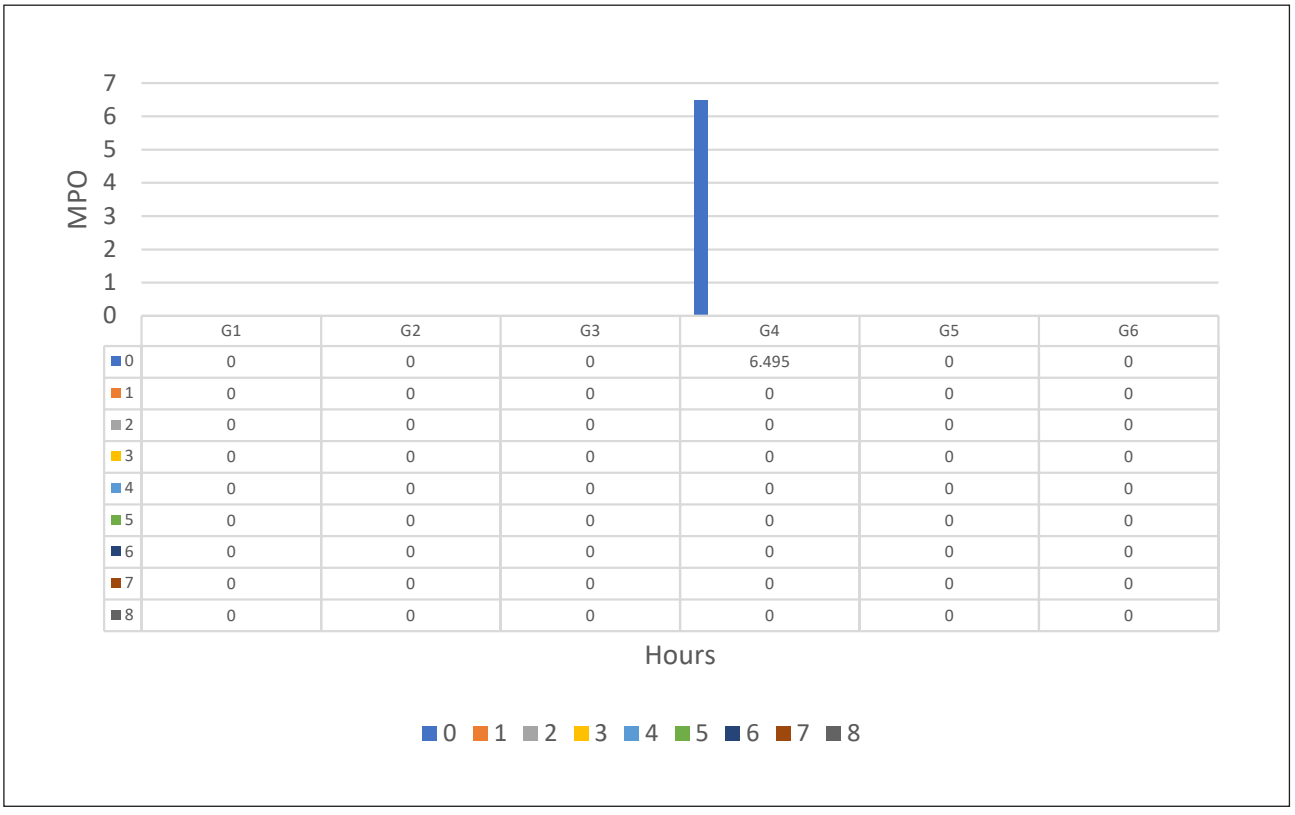
	G1	G2	G3	G4	G5	G6	G7	G8
0	8	14	10	19	15	18	12	18
1	10	0	26	19	14	11	14	17
2	9	0	25.5	16	16	18	13	19
3	10	18	16	19	19	15	12	16
4	0	12.5	29	20	15	11	14	18
5	11	17	45	26	21	20	20	18
6	13	25.5	96	32	31	39	24	20.5
7	18	23	14.5	39	37	36.5	22.5	27
8	18	30	14	58	53	51.5	33	32.5

SUPPLEMENTARY FIGURE 16. Group F: IL-6 levels at the designated times of the study.



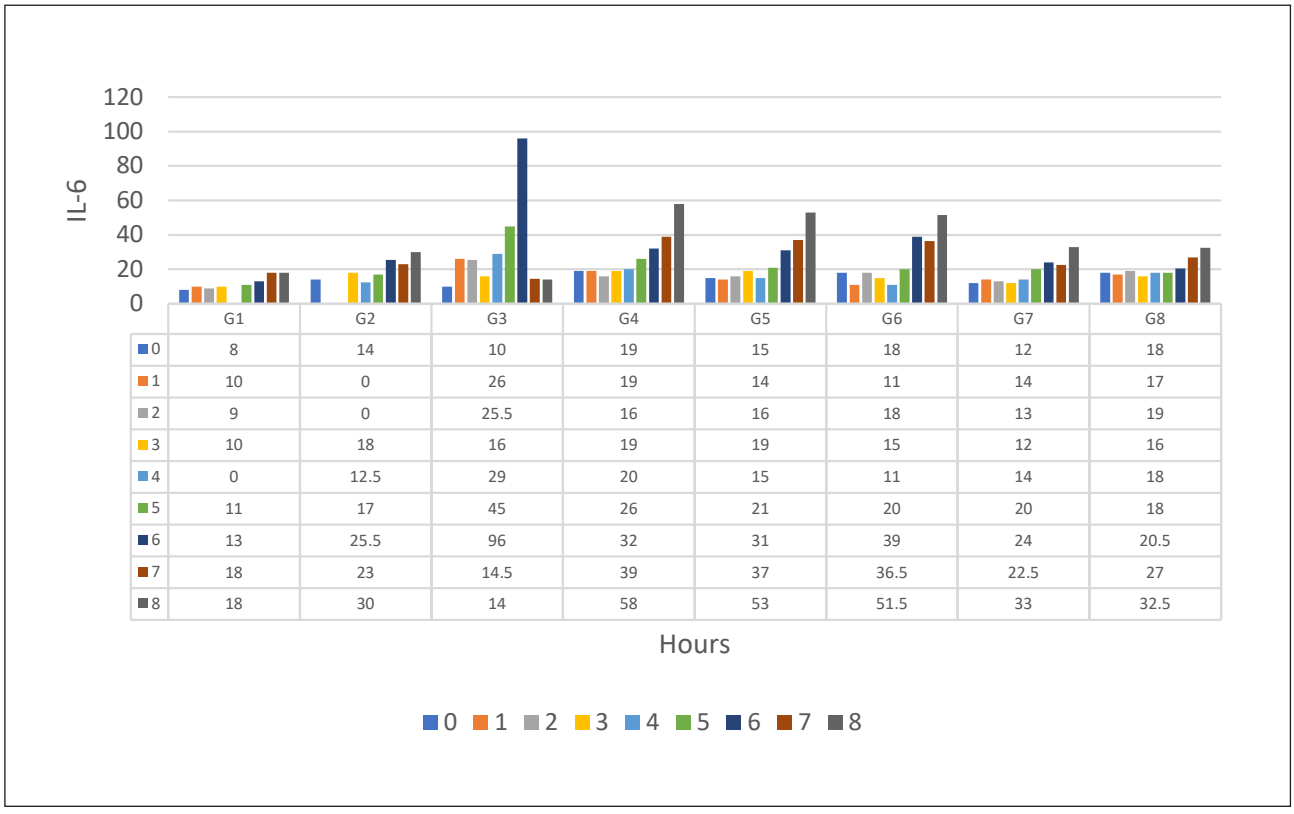
	G1	G2	G3	G4	G5	G6	G7	G8
0	4	6	3	5	3	4	6	4
1	4	0	12	6	4	4	4	4.5
2	4	0	13	3	4	5	5	4
3	4	6	7	4.5	7	4	6	5
4	0	5.5	10	4	7	3	5	7
5	7	6	19	5	16	5	36.5	8
6	7	15	8	6	8	6	24	7
7	11	5	8	6	7	4	9	6
8	9	5	7.5	4	4	4	12.5	5.5

SUPPLEMENTARY FIGURE 17. Group F: IL-10 levels at the designated times of the study.



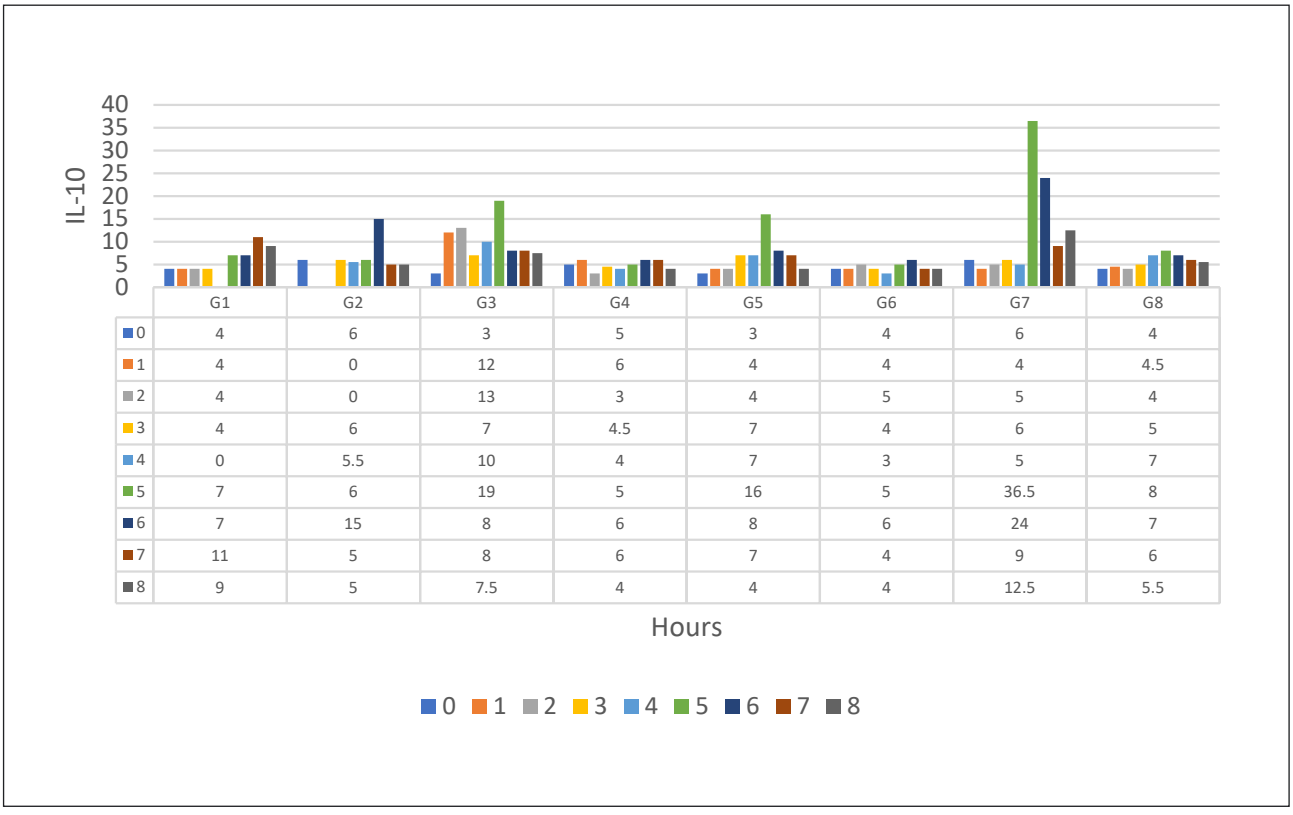
	G1	G2	G3	G4	G5	G6
0	0	0	0	6.495	0	0
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0

SUPPLEMENTARY FIGURE 18. Group F: MPO levels at the designated times of the study.



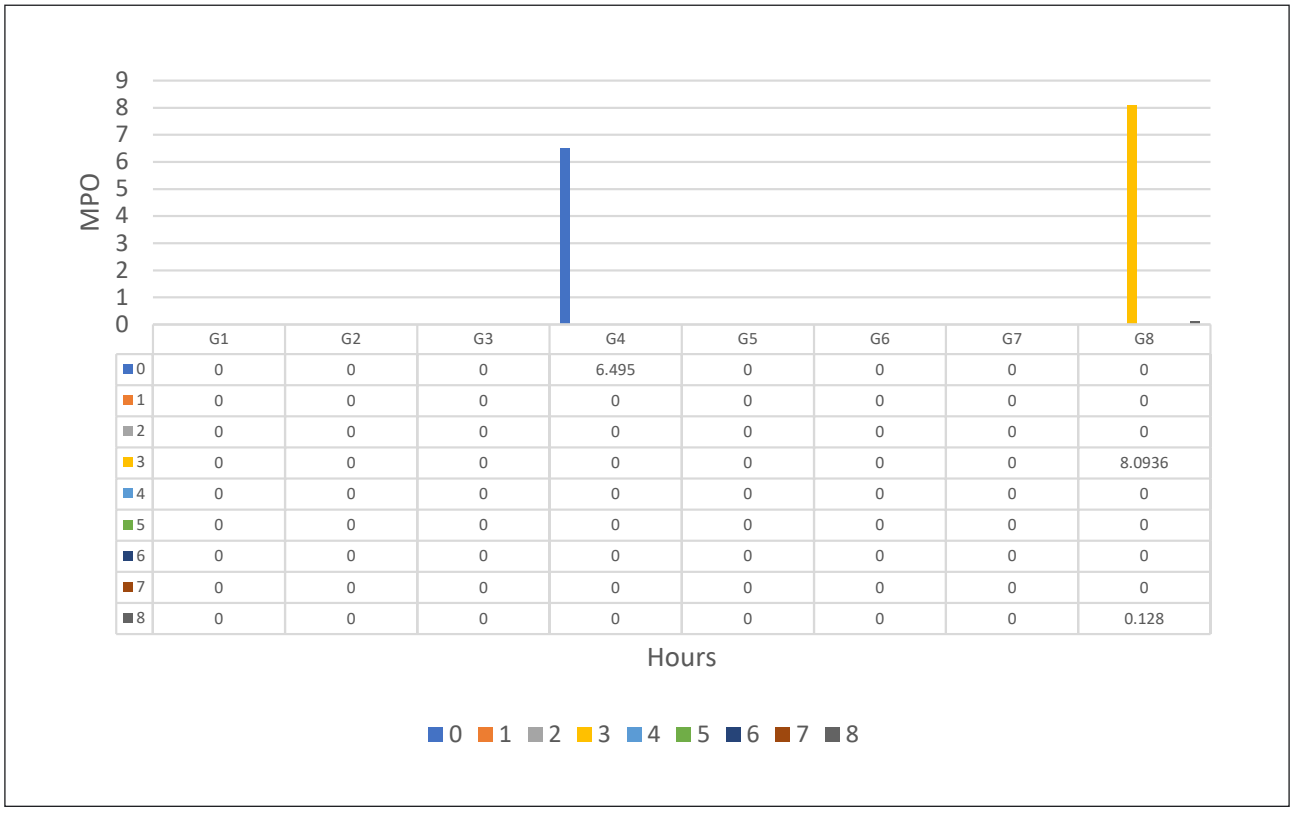
	G1	G2	G3	G4	G5	G6	G7	G8
0	8	14	10	19	15	18	12	18
1	10	0	26	19	14	11	14	17
2	9	0	25.5	16	16	18	13	19
3	10	18	16	19	19	15	12	16
4	0	12.5	29	20	15	11	14	18
5	11	17	45	26	21	20	20	18
6	13	25.5	96	32	31	39	24	20.5
7	18	23	14.5	39	37	36.5	22.5	27
8	18	30	14	58	53	51.5	33	32.5

SUPPLEMENTARY FIGURE 19. Group G: IL-6 levels at the designated times of the study.



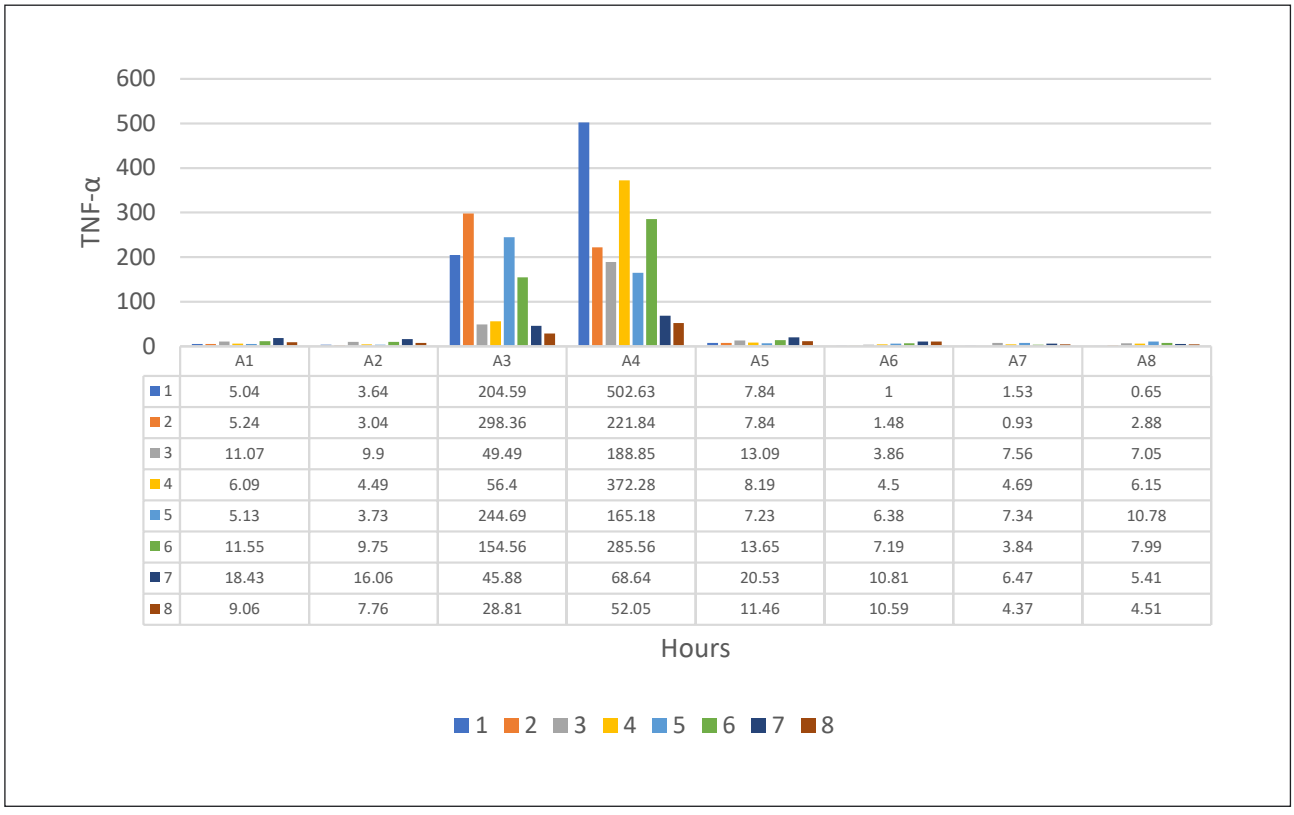
	G1	G2	G3	G4	G5	G6	G7	G8
0	4	6	3	5	3	4	6	4
1	4	0	12	6	4	4	4	4.5
2	4	0	13	3	4	5	5	4
3	4	6	7	4.5	7	4	6	5
4	0	5.5	10	4	7	3	5	7
5	7	6	19	5	16	5	36.5	8
6	7	15	8	6	8	6	24	7
7	11	5	8	6	7	4	9	6
8	9	5	7.5	4	4	4	12.5	5.5

SUPPLEMENTARY FIGURE 20. Group G: IL-10 levels at the designated times of the study.



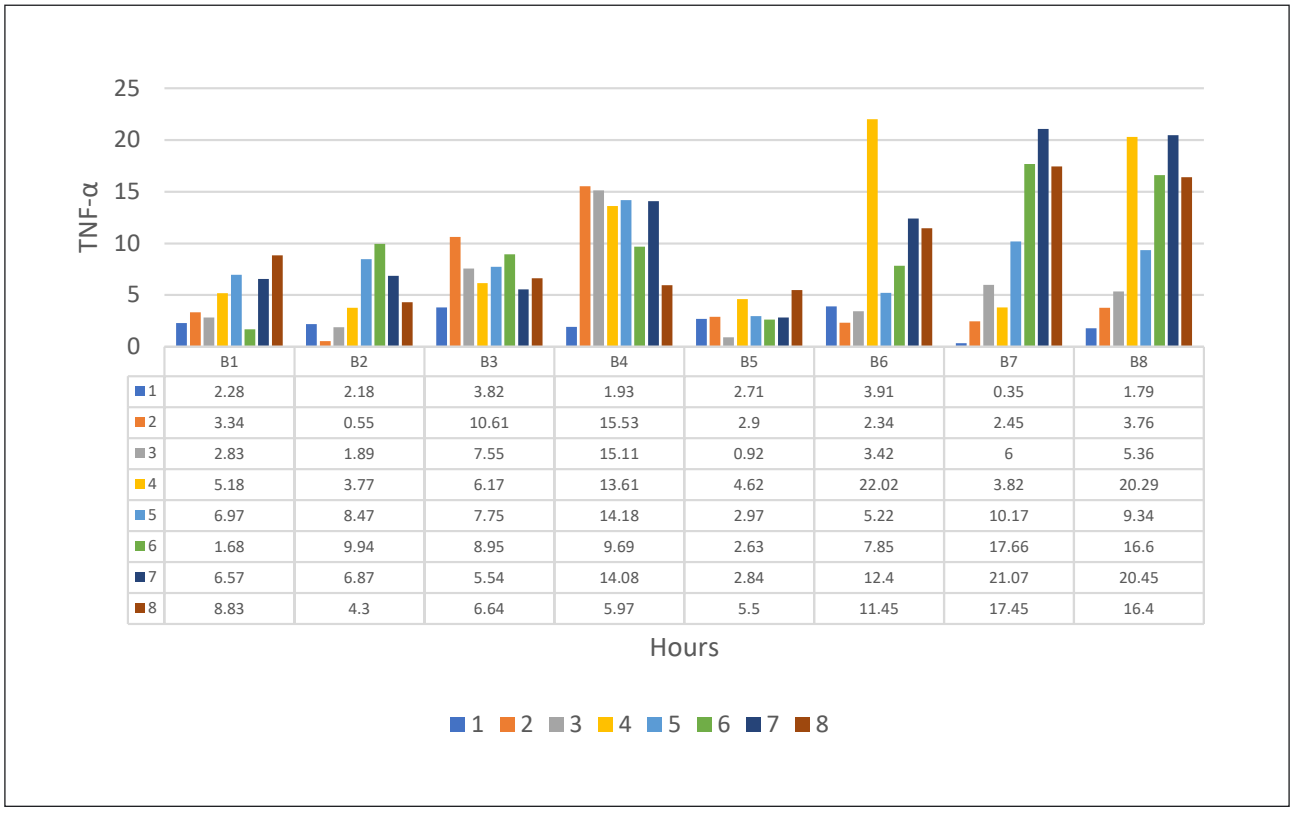
	G1	G2	G3	G4	G5	G6	G7	G8
0	0	0	0	6.495	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	8.0936
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0.128

SUPPLEMENTARY FIGURE 21. Group G: MPO levels at the designated times of the study.



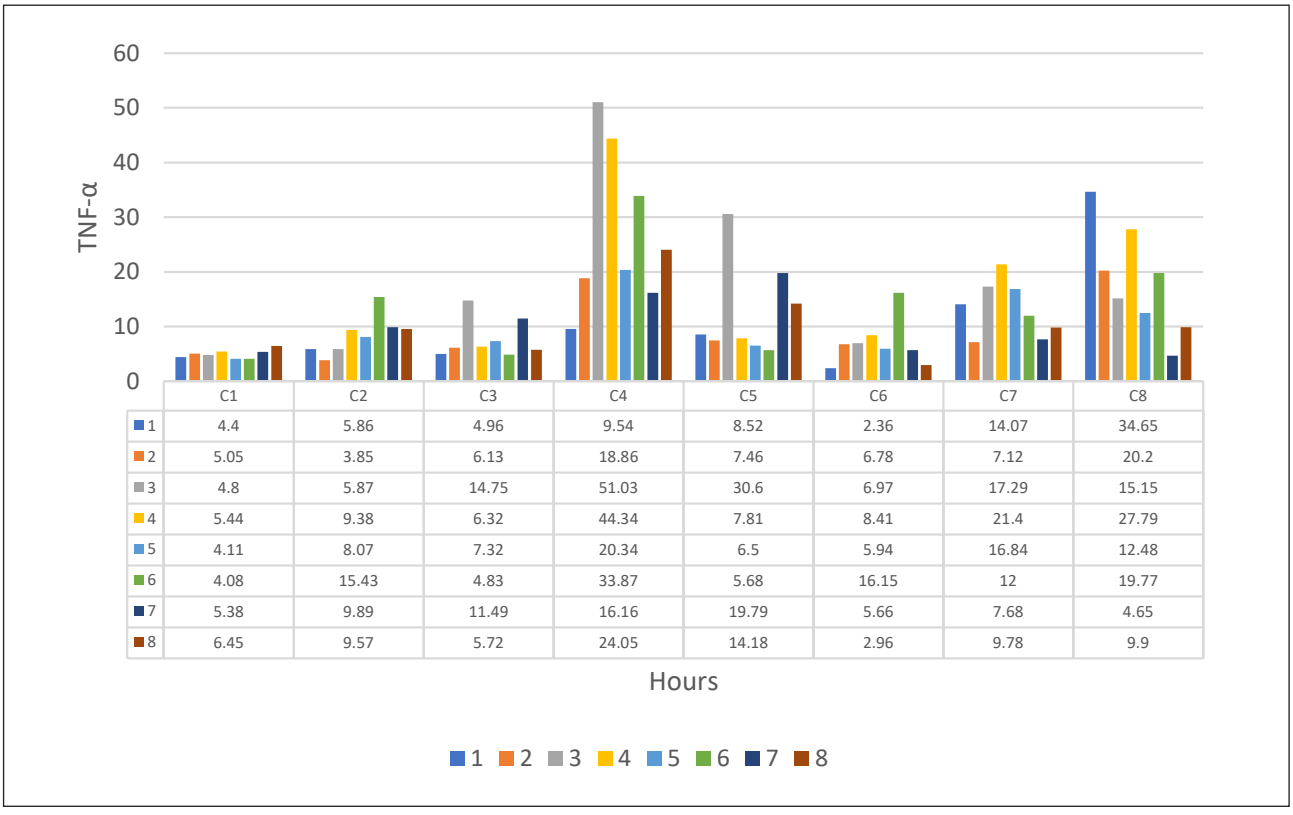
	A1	A2	A3	A4	A5	A6	A7	A8
1	5.04	3.64	204.59	502.63	7.84	1	1.53	0.65
2	5.24	3.04	298.36	221.84	7.84	1.48	0.93	2.88
3	11.07	9.9	49.49	188.85	13.09	3.86	7.56	7.05
4	6.09	4.49	56.4	372.28	8.19	4.5	4.69	6.15
5	5.13	3.73	244.69	165.18	7.23	6.38	7.34	10.78
6	11.55	9.75	154.56	285.56	13.65	7.19	3.84	7.99
7	18.43	16.06	45.88	68.64	20.53	10.81	6.47	5.41
8	9.06	7.76	28.81	52.05	11.46	10.59	4.37	4.51

SUPPLEMENTARY FIGURE 22. Group A: TNF-α levels at the control group.



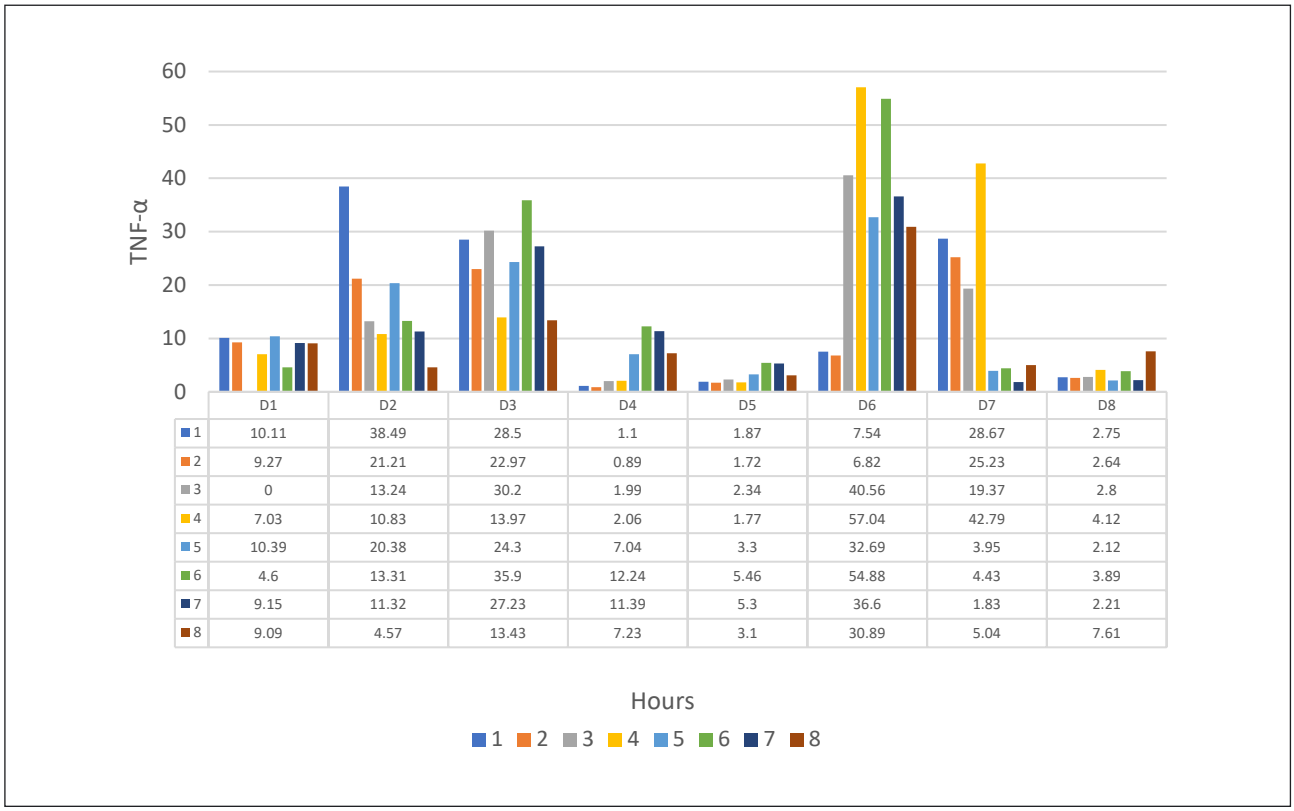
	B1	B2	B3	B4	B5	B6	B7	B8
1	2.28	2.18	3.82	1.93	2.71	3.91	0.35	1.79
2	3.34	0.55	10.61	15.53	2.9	2.34	2.45	3.76
3	2.83	1.89	7.55	15.11	0.92	3.42	6	5.36
4	5.18	3.77	6.17	13.61	4.62	22.02	3.82	20.29
5	6.97	8.47	7.75	14.18	2.97	5.22	10.17	9.34
6	1.68	9.94	8.95	9.69	2.63	7.85	17.66	16.6
7	6.57	6.87	5.54	14.08	2.84	12.4	21.07	20.45
8	8.83	4.3	6.64	5.97	5.5	11.45	17.45	16.4

SUPPLEMENTARY FIGURE 23. Group B: TNF-α levels at the Study group which Lazaroid was given at 30 minutesofacute myocardial ischemia.



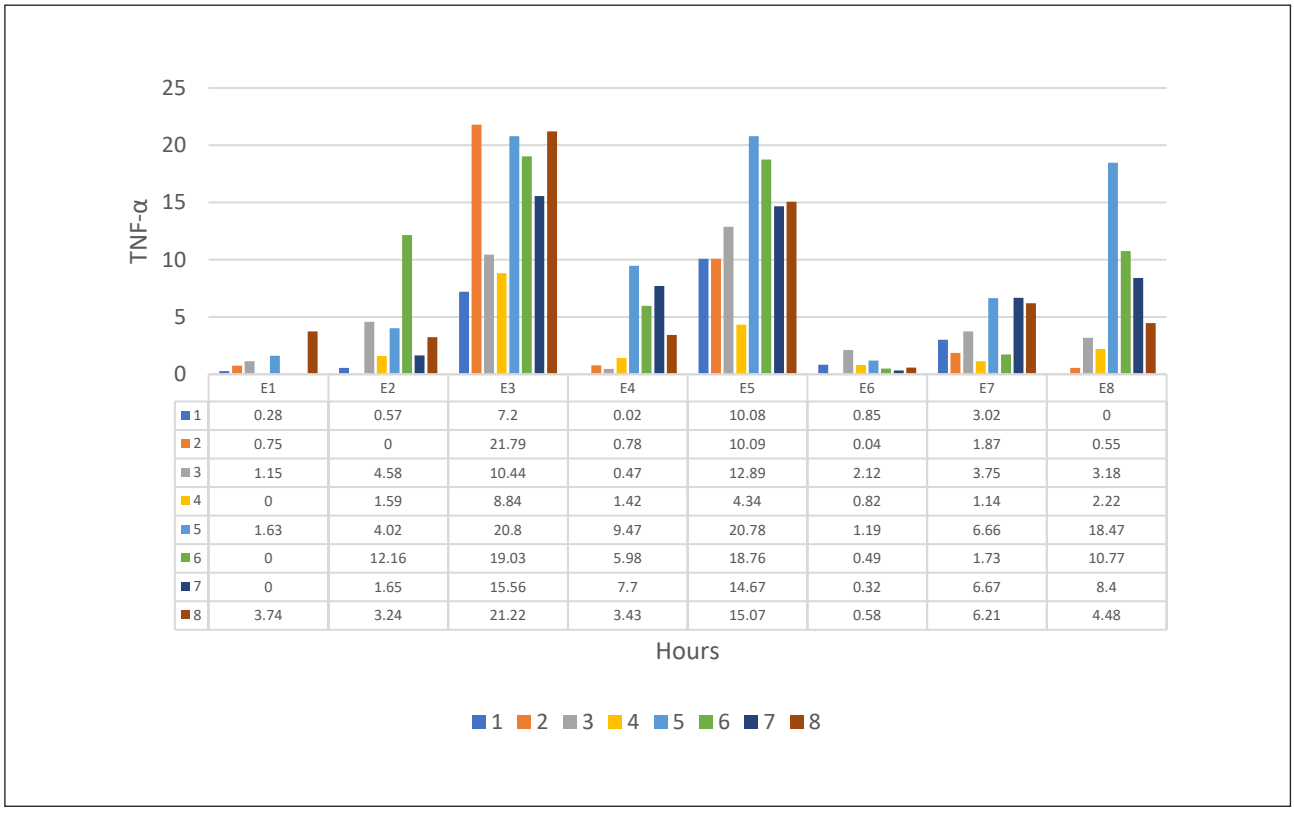
	C1	C2	C3	C4	C5	C6	C7	C8
1	4.4	5.86	4.96	9.54	8.52	2.36	14.07	34.65
2	5.05	3.85	6.13	18.86	7.46	6.78	7.12	20.2
3	4.8	5.87	14.75	51.03	30.6	6.97	17.29	15.15
4	5.44	9.38	6.32	44.34	7.81	8.41	21.4	27.79
5	4.11	8.07	7.32	20.34	6.5	5.94	16.84	12.48
6	4.08	15.43	4.83	33.87	5.68	16.15	12	19.77
7	5.38	9.89	11.49	16.16	19.79	5.66	7.68	4.65
8	6.45	9.57	5.72	24.05	14.18	2.96	9.78	9.9

SUPPLEMENTARY FIGURE 24. Group C: TNF-α levelsat the Study group which Lazaroid was given before acute myocardial ischemia.



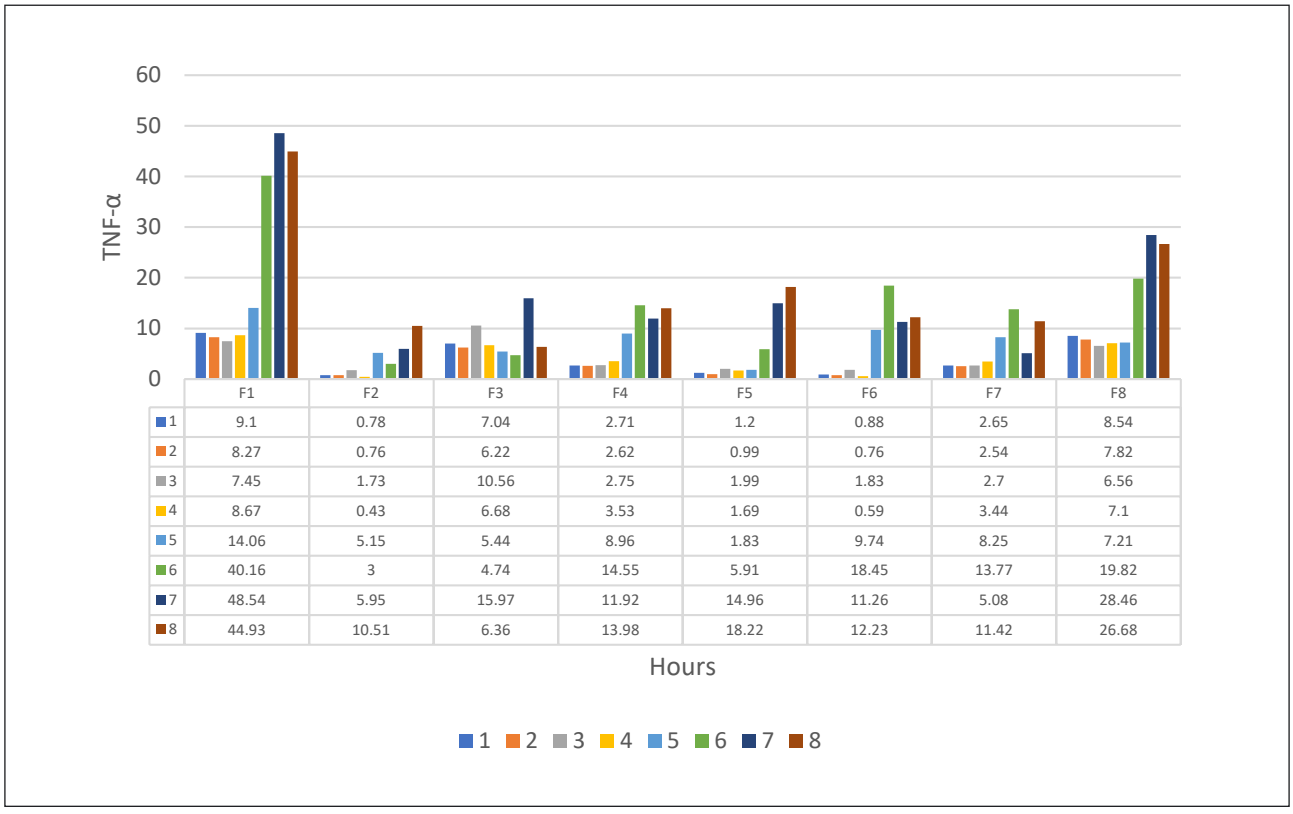
	D1	D2	D3	D4	D5	D6	D7	D8
1	10.11	38.49	28.5	1.1	1.87	7.54	28.67	2.75
2	9.27	21.21	22.97	0.89	1.72	6.82	25.23	2.64
3	0	13.24	30.2	1.99	2.34	40.56	19.37	2.8
4	7.03	10.83	13.97	2.06	1.77	57.04	42.79	4.12
5	10.39	20.38	24.3	7.04	3.3	32.69	3.95	2.12
6	4.6	13.31	35.9	12.24	5.46	54.88	4.43	3.89
7	9.15	11.32	27.23	11.39	5.3	36.6	1.83	2.21
8	9.09	4.57	13.43	7.23	3.1	30.89	5.04	7.61

SUPPLEMENTARY FIGURE 25. Group D: TNF-α levels at the Study group which Sildenafil was given at 30 minutes of acute myocardial ischemia.



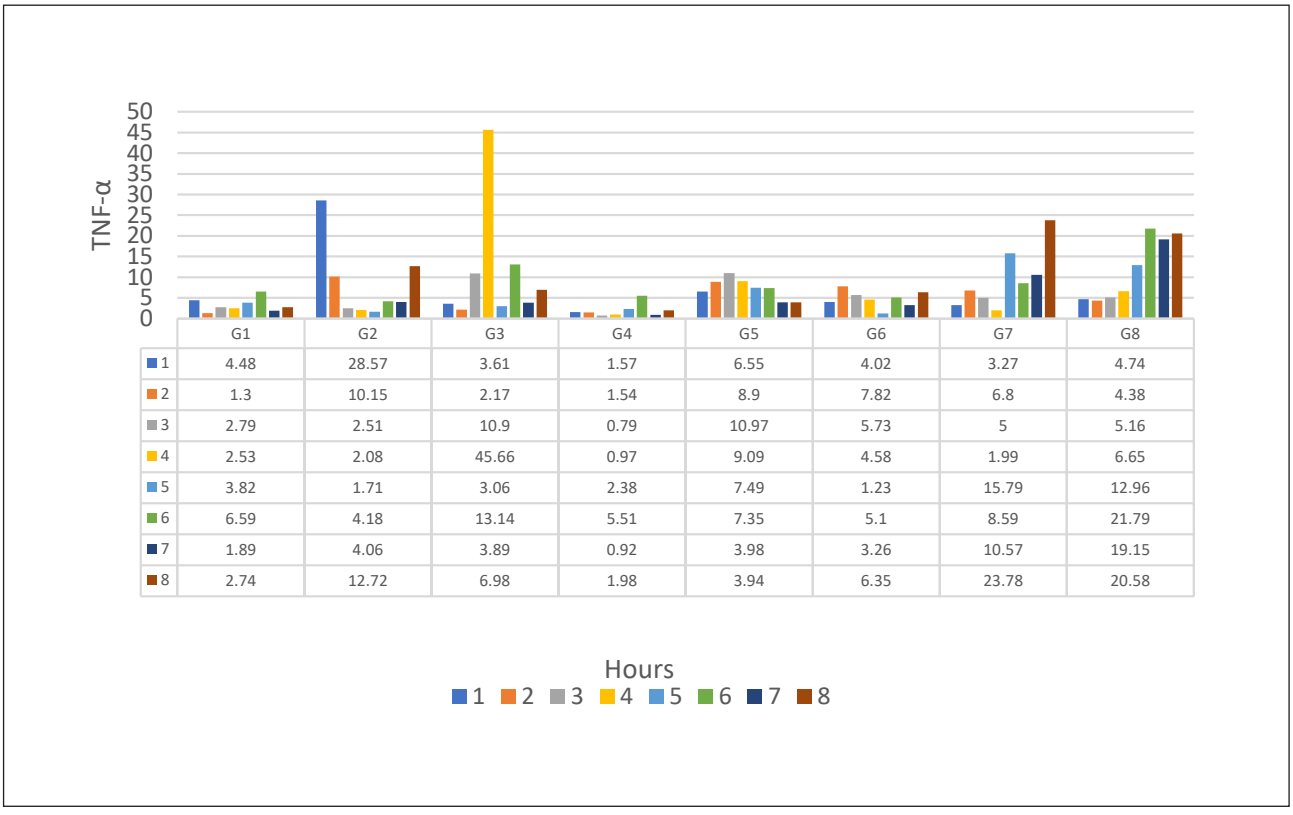
	E1	E2	E3	E4	E5	E6	E7	E8
1	0.28	0.57	7.2	0.02	10.08	0.85	3.02	0
2	0.75	0	21.79	0.78	10.09	0.04	1.87	0.55
3	1.15	4.58	10.44	0.47	12.89	2.12	3.75	3.18
4	0	1.59	8.84	1.42	4.34	0.82	1.14	2.22
5	1.63	4.02	20.8	9.47	20.78	1.19	6.66	18.47
6	0	12.16	19.03	5.98	18.76	0.49	1.73	10.77
7	0	1.65	15.56	7.7	14.67	0.32	6.67	8.4
8	3.74	3.24	21.22	3.43	15.07	0.58	6.21	4.48

SUPPLEMENTARY FIGURE 26. Group E: TNF-α levels at the Study group which Sildenafil was given before myocardial ischemia.



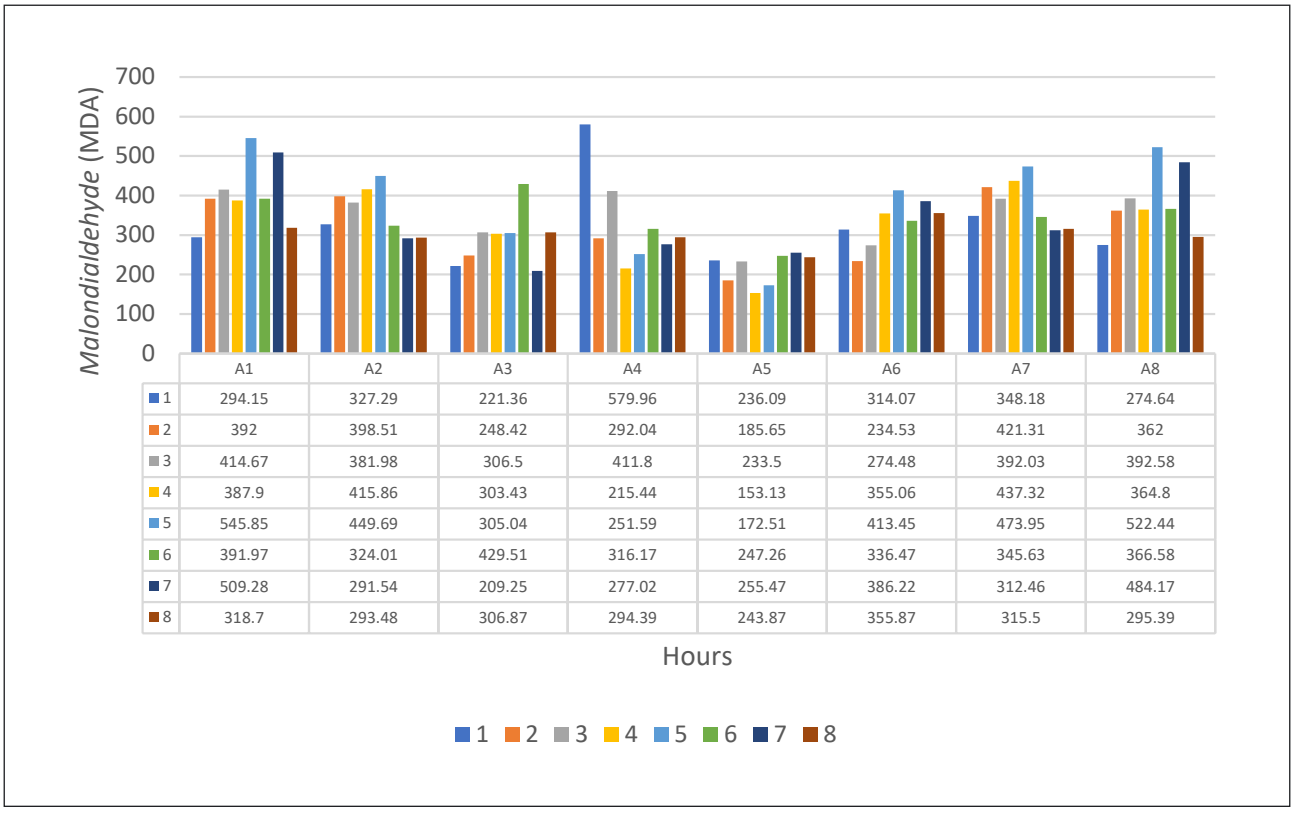
	F1	F2	F3	F4	F5	F6	F7	F8
1	9.1	0.78	7.04	2.71	1.2	0.88	2.65	8.54
2	8.27	0.76	6.22	2.62	0.99	0.76	2.54	7.82
3	7.45	1.73	10.56	2.75	1.99	1.83	2.7	6.56
4	8.67	0.43	6.68	3.53	1.69	0.59	3.44	7.1
5	14.06	5.15	5.44	8.96	1.83	9.74	8.25	7.21
6	40.16	3	4.74	14.55	5.91	18.45	13.77	19.82
7	48.54	5.95	15.97	11.92	14.96	11.26	5.08	28.46
8	44.93	10.51	6.36	13.98	18.22	12.23	11.42	26.68

SUPPLEMENTARY FIGURE 27. Group F: TNF-α levelsat theStudy group which Combination sildenafiland lazaroid was given at 30 minutes of acute myocardialischemia.



	G1	G2	G3	G4	G5	G6	G7	G8
1	4.48	28.57	3.61	1.57	6.55	4.02	3.27	4.74
2	1.3	10.15	2.17	1.54	8.9	7.82	6.8	4.38
3	2.79	2.51	10.9	0.79	10.97	5.73	5	5.16
4	2.53	2.08	45.66	0.97	9.09	4.58	1.99	6.65
5	3.82	1.71	3.06	2.38	7.49	1.23	15.79	12.96
6	6.59	4.18	13.14	5.51	7.35	5.1	8.59	21.79
7	1.89	4.06	3.89	0.92	3.98	3.26	10.57	19.15
8	2.74	12.72	6.98	1.98	3.94	6.35	23.78	20.58

SUPPLEMENTARY FIGURE 28. Group G: TNF-α levels at the Studygroup which Combination sildenafiland lazaroid was given before myocardial ischemia.

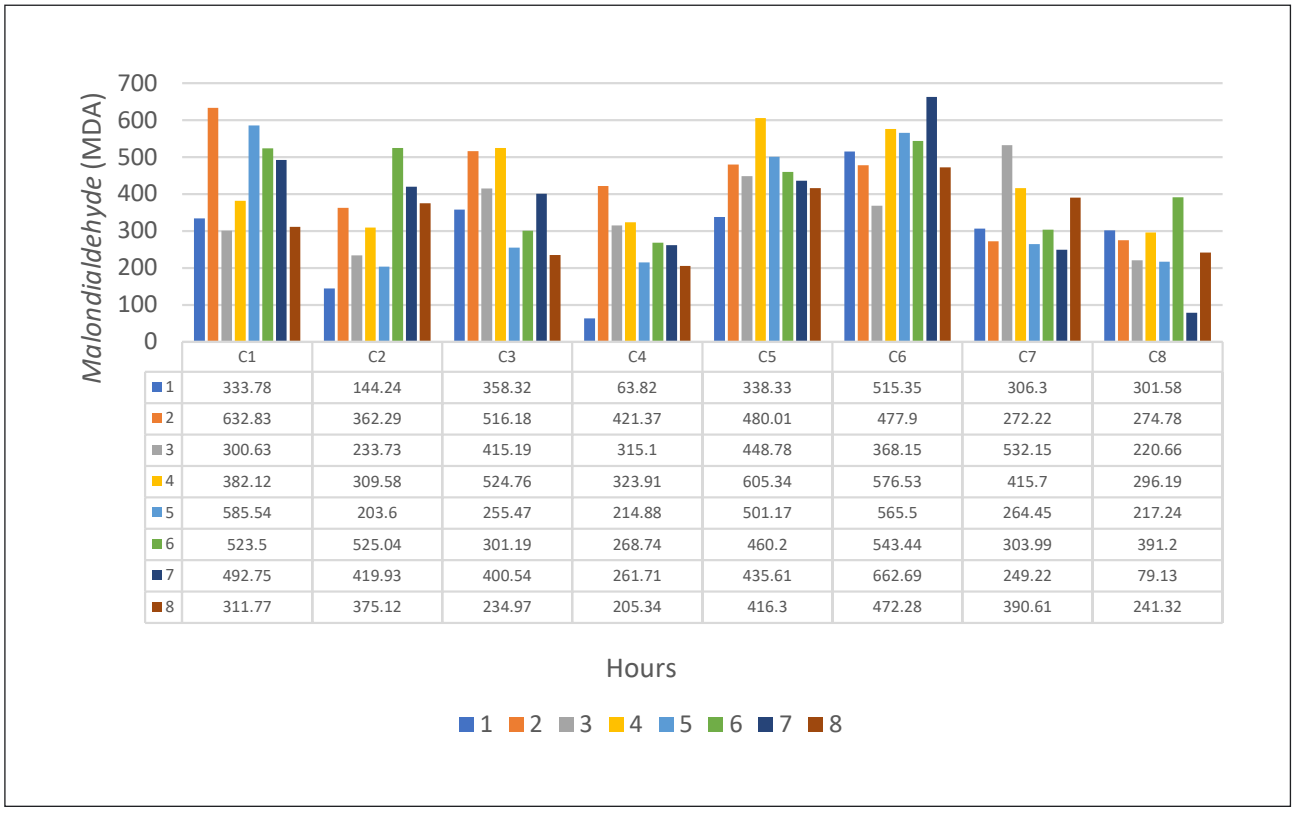


	A1	A2	A3	A4	A5	A6	A7	A8
1	294.15	327.29	221.36	579.96	236.09	314.07	348.18	274.64
2	392	398.51	248.42	292.04	185.65	234.53	421.31	362
3	414.67	381.98	306.5	411.8	233.5	274.48	392.03	392.58
4	387.9	415.86	303.43	215.44	153.13	355.06	437.32	364.8
5	545.85	449.69	305.04	251.59	172.51	413.45	473.95	522.44
6	391.97	324.01	429.51	316.17	247.26	336.47	345.63	366.58
7	509.28	291.54	209.25	277.02	255.47	386.22	312.46	484.17
8	318.7	293.48	306.87	294.39	243.87	355.87	315.5	295.39

SUPPLEMENTARY FIGURE 29. Group A: *Malondialdehyde* (MDA) levels at the control group.

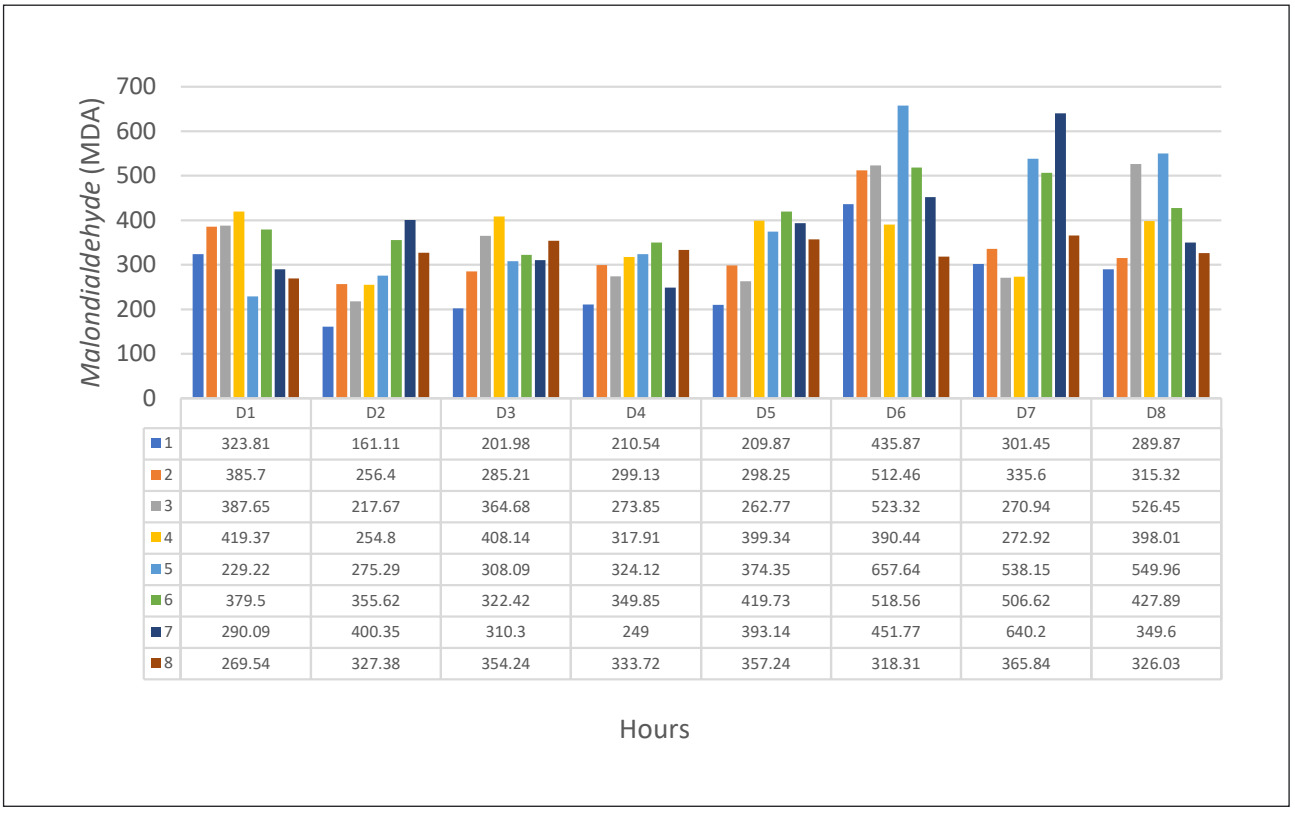
	B1	B2	B3	B4	B5	B6	B7	B8
1	251.38	357.75	217.35	324.82	402.57	64.87	92.44	82.49
2	359.51	363.05	297.5	171.92	240.54	91.02	276.8	136.68
3	304.76	426.35	228.2	189.11	256.44	177.06	258.17	133.2
4	186.61	367.39	226.14	175.69	235.96	348.12	252.15	111.21
5	193.04	409.12	263.89	347.7	262.05	408.89	315.65	96.51
6	293.32	562.4	216.46	256.73	195.29	461.81	207.29	175.27
7	244.38	482.98	298.13	332.68	241	491.91	202.21	113.24
8	237.3	200.96	315.46	287.53	194.86	493.97	230.21	106.78

SUPPLEMENTARY FIGURE 30. Group B: *Malondialdehyde* (MDA) levels at the Study group which Lazaroid was given at 30 minutes of acutemyocardial ischemia.



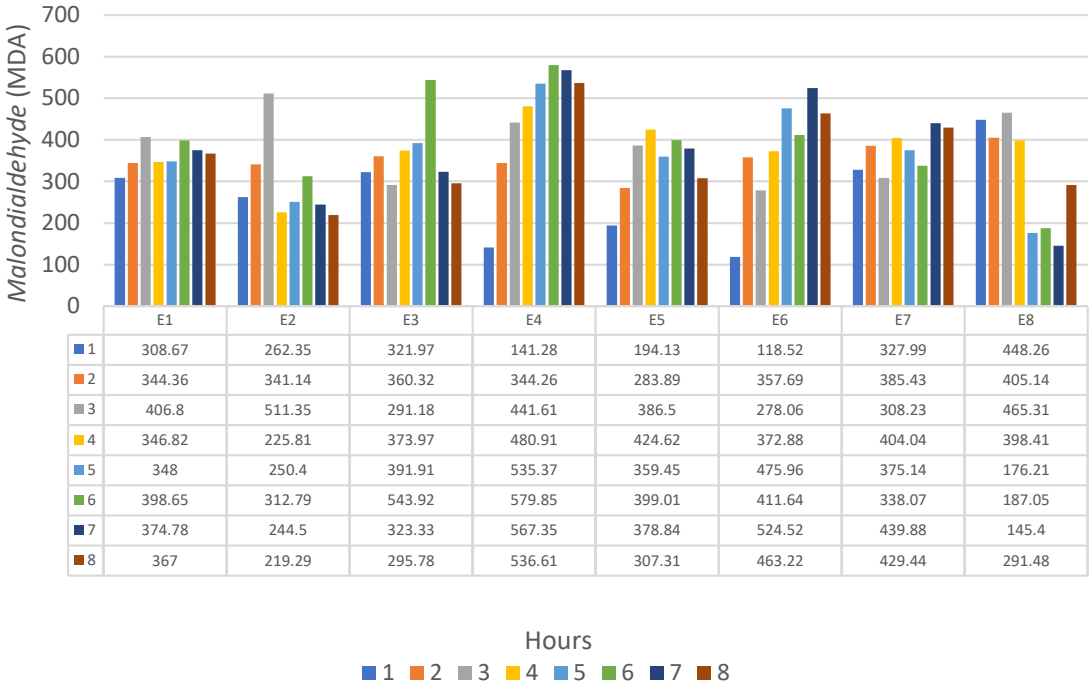
	C1	C2	C3	C4	C5	C6	C7	C8
1	333.78	144.24	358.32	63.82	338.33	515.35	306.3	301.58
2	632.83	362.29	516.18	421.37	480.01	477.9	272.22	274.78
3	300.63	233.73	415.19	315.1	448.78	368.15	532.15	220.66
4	382.12	309.58	524.76	323.91	605.34	576.53	415.7	296.19
5	585.54	203.6	255.47	214.88	501.17	565.5	264.45	217.24
6	523.5	525.04	301.19	268.74	460.2	543.44	303.99	391.2
7	492.75	419.93	400.54	261.71	435.61	662.69	249.22	79.13
8	311.77	375.12	234.97	205.34	416.3	472.28	390.61	241.32

SUPPLEMENTARY FIGURE 31. Group C: *Malondialdehyde* (MDA) levels at the Study group which Lazaroid was given before acute myocardialischemia.



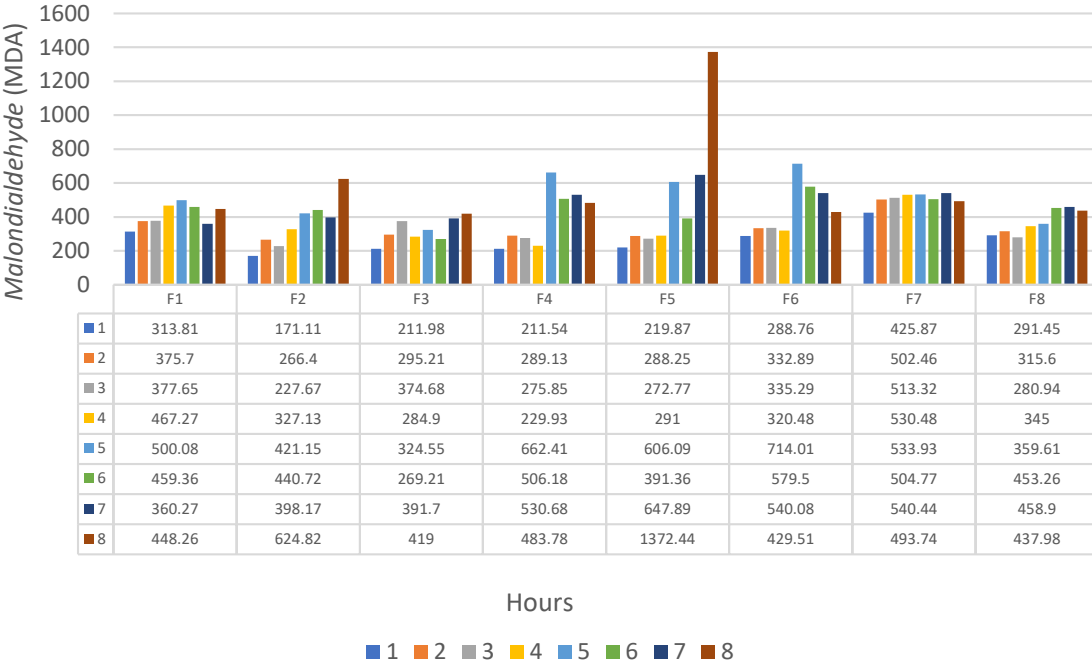
	D1	D2	D3	D4	D5	D6	D7	D8
1	323.81	161.11	201.98	210.54	209.87	435.87	301.45	289.87
2	385.7	256.4	285.21	299.13	298.25	512.46	335.6	315.32
3	387.65	217.67	364.68	273.85	262.77	523.32	270.94	526.45
4	419.37	254.8	408.14	317.91	399.34	390.44	272.92	398.01
5	229.22	275.29	308.09	324.12	374.35	657.64	538.15	549.96
6	379.5	355.62	322.42	349.85	419.73	518.56	506.62	427.89
7	290.09	400.35	310.3	249	393.14	451.77	640.2	349.6
8	269.54	327.38	354.24	333.72	357.24	318.31	365.84	326.03

SUPPLEMENTARY FIGURE 32. Group D: *Malondialdehyde* (MDA) levels at the Study group which Sildenafil was given at 30 minutes of acutemyocardial ischemia.



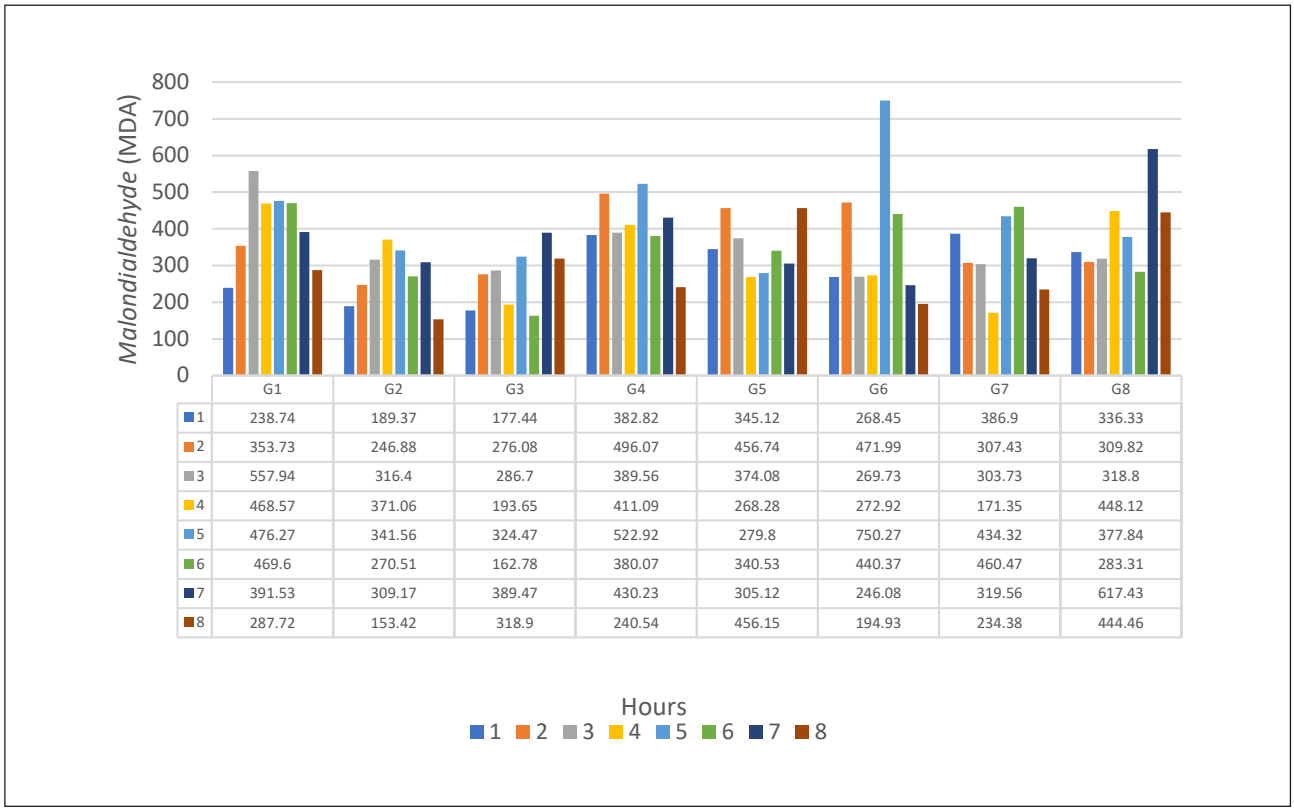
	E1	E2	E3	E4	E5	E6	E7	E8
1	308.67	262.35	321.97	141.28	194.13	118.52	327.99	448.26
2	344.36	341.14	360.32	344.26	283.89	357.69	385.43	405.14
3	406.8	511.35	291.18	441.61	386.5	278.06	308.23	465.31
4	346.82	225.81	373.97	480.91	424.62	372.88	404.04	398.41
5	348	250.4	391.91	535.37	359.45	475.96	375.14	176.21
6	398.65	312.79	543.92	579.85	399.01	411.64	338.07	187.05
7	374.78	244.5	323.33	567.35	378.84	524.52	439.88	145.4
8	367	219.29	295.78	536.61	307.31	463.22	429.44	291.48

SUPPLEMENTARY FIGURE 33. Group E: *Malondialdehyde* (MDA) levels at the Study group which Sildenafil was given before myocardial ischemia.



	F1	F2	F3	F4	F5	F6	F7	F8
1	313.81	171.11	211.98	211.54	219.87	288.76	425.87	291.45
2	375.7	266.4	295.21	289.13	288.25	332.89	502.46	315.6
3	377.65	227.67	374.68	275.85	272.77	335.29	513.32	280.94
4	467.27	327.13	284.9	229.93	291	320.48	530.48	345
5	500.08	421.15	324.55	662.41	606.09	714.01	533.93	359.61
6	459.36	440.72	269.21	506.18	391.36	579.5	504.77	453.26
7	360.27	398.17	391.7	530.68	647.89	540.08	540.44	458.9
8	448.26	624.82	419	483.78	1372.44	429.51	493.74	437.98

SUPPLEMENTARY FIGURE 34. Group F: *Malondialdehyde* (MDA) levels at the Studygroup which Combination sildenafil and lazaroid was given at 30 minutes of acutemyocardial ischemia.



	G1	G2	G3	G4	G5	G6	G7	G8
1	238.74	189.37	177.44	382.82	345.12	268.45	386.9	336.33
2	353.73	246.88	276.08	496.07	456.74	471.99	307.43	309.82
3	557.94	316.4	286.7	389.56	374.08	269.73	303.73	318.8
4	468.57	371.06	193.65	411.09	268.28	272.92	171.35	448.12
5	476.27	341.56	324.47	522.92	279.8	750.27	434.32	377.84
6	469.6	270.51	162.78	380.07	340.53	440.37	460.47	283.31
7	391.53	309.17	389.47	430.23	305.12	246.08	319.56	617.43
8	287.72	153.42	318.9	240.54	456.15	194.93	234.38	444.46

SUPPLEMENTARY FIGURE 35. Group G: *Malondialdehyde* (MDA) levels at the Study group which Combination sildenafil and lazaroid was given before myocardialischemia.