Effectiveness of ice bath therapy after high-intensity exercise in reducing inflammation, oxidative stress, and creatine kinase activity in adolescent males

La eficacia de la terapia de baño de hielo después del ejercicio de alta intensidad reduce los niveles de inflamación, el estrés oxidativo y la actividad de la creatina quinasa en varones adolescentes

Authors

Adi Pranoto [1]
Irmantara Subagio [1]
Gigih Siantoro [1]
Afif Rusdiawan [1]
Mochamad Purnomo [1]
Donny Ardy Kusuma [1]
Bhekti Lestari [1]
Muhammad Kharis Fajar [1]
I Dewa Made Aryananda Wijaya Kusuma [1]
Bekir Erhan Orhan [2]

[1] Universitas Negeri Surabaya (Indonesia)
[2] Istanbul Aydın University (Turkey)

Corresponding author:
Afif Rusdiawan
afifrusdiawan@unesa.ac.id

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Abstract

Introduction: Recovery is a vital component of physical activity, serving to restore the body's physiological equilibrium after intense exertion. However, effective and efficient models of recovery therapy remain underreported in current literature.
Objective: This quasi-experimental study aimed to examine the effects of ice bath therapy following high-intensity exercise on markers of inflammation, oxidative stress, and creatine kinase (CK) activity in adolescent males.
Methodology: Twenty male undergraduate students (n = 20; aged 19–22 years) majoring in Football Coaching at the Faculty of Sports and Health Sciences, Universitas Negeri Surabaya (UNESA), participated in this study. They received ice bath therapy (IBT) after high-intensity exercise for four weeks. IL-6 concentrations were measured using an ELISA Kit, MDA levels with a Colorimetric Assay Kit, and CK activity with an Activity Assay Kit. Paired and independent sample t-tests were used, and Cohen's d was calculated to determine effect size at a 95% confidence level.
Results: Significant reductions were observed in Interleukin-6 (IL-6) by 29.49 %, Malondialdehyde (MDA) by 37.75 %, and CK activity by 84.45 % after intervention in the IBT group (all $p \leq 0.05$). Between-group comparisons also revealed significant differences in all three biomarkers (all $p \leq 0.05$), with large to very large effect sizes (Cohen's $d > 1.7$), indicating a strong impact of the therapy.
Conclusions: Ice bath therapy after high-intensity exercise can be an effective therapy model in significantly reducing levels of IL-6, MDA, and CK activity in adolescent males.

Keywords

High-intensity exercise; ice bath therapy; recovery mode; soccer athlete.

Resumen

Introducción: La recuperación es un componente vital de la actividad física, ya que sirve para restablecer el equilibrio fisiológico del cuerpo tras un esfuerzo intenso. Sin embargo, en la literatura actual, aún no se han publicado modelos eficaces y eficientes de terapia de recuperación.
Objetivo: Este estudio cuasi-experimental tuvo como objetivo examinar los efectos de la terapia con baño de hielo después del ejercicio de alta intensidad sobre los marcadores de inflamación, estrés oxidativo y actividad de la creatina quinasa (CK) en varones adolescentes.
Metodología: Participaron veinte estudiantes universitarios varones (n = 20; entre 19 y 22 años) del programa de Formación en Fútbol de la Facultad de Ciencias del Deporte y la Salud de la Universidad Estatal de Surabaya (UNESA). Recibieron terapia con baño de hielo (IBT) después del ejercicio intenso durante cuatro semanas. Las concentraciones de IL-6 se midieron con un kit ELISA, los niveles de MDA con un kit colorimétrico y la actividad de CK con un kit de ensayo de actividad. Se utilizaron pruebas t para muestras apareadas e independientes y se calculó el tamaño del efecto (Cohen's d) con un nivel de confianza del 95 %.
Resultados: Se observaron reducciones significativas en IL-6 (29.49 %), MDA (37.75 %) y actividad de CK (84.45 %) tras la intervención en el grupo IBT (todos $p \leq 0{,}005$). También se observaron diferencias significativas entre grupos en los tres biomarcadores (todos $p \leq 0{,}05$), con tamaños del efecto grandes a muy grandes (Cohen's $d > 1{,}7$), lo que indica un fuerte impacto de la terapia.
Conclusiones: La terapia de baño de hielo después del ejercicio de alta intensidad puede ser un modelo terapéutico eficaz para reducir significativamente los niveles de actividad de IL-6, MDA y CK en varones adolescentes.

Palabras clave

Ejercicio de alta intensidad; terapia de baño de hielo; modo de recuperación; atleta de fútbol.

Introduction

High-intensity interval training (HIIT) has become increasingly popular among adolescents, in line with growing trends in fitness and competitive sports (Bond et al., 2017; Ma et al., 2024). This form of exercise is widely adopted for its effectiveness in improving cardiovascular function, muscular strength, and overall metabolic health (Garber et al., 2011; Weston et al., 2014). However, high-intensity exercise also poses physiological risks. The mechanical and metabolic demands of such exertion can result in exercise-induced muscle damage (Leite et al., 2023; Andrews et al., 2024), which triggers an inflammatory response as part of the body's natural repair processes (Krüger & Mooren, 2014). Additionally, elevated levels of reactive oxygen species (ROS) during intense activity may surpass the body's antioxidant defense capacity, causing oxidative stress and further cellular damage (Pingitore et al., 2015). Adolescents may be particularly vulnerable to these responses due to ongoing physiological development, including immature antioxidant systems, hormonal fluctuations, and less efficient thermoregulation mechanisms (Faigenbaum & Myer, 2009; Peake, 2019). These developmental characteristics can amplify inflammation and oxidative damage following intense exercise. Understanding these physiological responses is crucial for the development of effective recovery strategies, especially for adolescents engaged in frequent high-intensity training.

Recovery is a critical aspect of physical activity, enabling the restoration of physiological balance following strenuous exercise (Peake, 2019). In recent years, running and other forms of exercise have gained popularity (Lee et al., 2017), particularly among individuals who favor time-efficient methods such as HIIT (Lee et al., 2024; Štajer et al., 2022). The appeal of HIIT lies in its ability to deliver measurable health and fitness improvements within a shorter duration. However, without proper recovery, the intensity of such training may lead to negative physiological outcomes or reduce motivation due to increased fatigue or discomfort. Post-exercise recovery is a multifaceted (physiological and psychological) process that is essential for sustaining performance and facilitating long-term adaptation (Hottenrott et al., 2021; Kellmann et al., 2018; Mujika, 2017). Various structured approaches have been introduced to support effective recovery, including cryotherapy.

Given the high physiological demands of running-based high-intensity exercise, recovery strategies are essential to ensure optimal adaptation and to prevent overtraining. Intense running can result in muscle damage and activate inflammatory and oxidative stress responses, which, if unaddressed, may impair subsequent performance. Cryotherapy, including cold-water immersion and localized ice application, has been employed to accelerate recovery after sprint-based activities, with reported benefits such as reduced delayed onset muscle soreness (DOMS), lower perceived fatigue, and decreased levels of muscle damage biomarkers (Hohenauer et al., 2015; Ihsan et al., 2021; Murray & Cardinale, 2015). Despite its increasing use, limited studies have explored how cold compression techniques affect specific biomarkers of muscle damage and inflammation, particularly interleukin-6 (IL-6), creatine kinase (CK), and malondialdehyde (MDA), following high-intensity exercise. Moreover, traditional recovery methods may not adequately manage fatigue in adolescent populations, who are more susceptible to overtraining due to ongoing physiological development. While cryotherapy has been widely studied in adult populations, limited research has explored its impact on adolescent athletes—especially in the context of specific biomarkers such as IL-6, MDA, and CK. Most existing studies focus on general recovery perceptions or muscle soreness rather than biochemical indicators of inflammation and oxidative stress. Furthermore, studies often lack controlled interventions tailored to younger populations. This study addresses these gaps by evaluating the biochemical efficacy of ice bath therapy in adolescent males undergoing structured high-intensity training. Therefore, this study aims to examine the effects of ice bath therapy following high-intensity exercise on levels of inflammation, creatine kinase, and oxidative stress in adolescent males.

Method

Study design

This research employed a quasi-experimental method using a two-group pretest–posttest design. A total of 20 male undergraduate students majoring in Football Coaching at the Faculty of Sports and Health

Sciences (FIKK), Universitas Negeri Surabaya (UNESA), aged between 19 and 22 years, with a normal BMI, a minimum of five years of football training experience, no history of injury, and no record of smoking or alcohol consumption in the past five years, were selected as research participants. Participants were randomly divided into two groups (n=10), namely the control group (CTR), and ice bath therapy (IBT). The sample size calculation used the Higgins & Kleinbaum (1985) formula with reference values from previous similar studies (Vaile et al., 2008) to obtain the minimum sample size (n=10) and 20 participants were taken for two groups. Informed consent was obtained from all participants before the study. All procedures applied in this research adhered to the ethical principles of the Declaration of Helsinki of the World Medical Association regarding research involving human subjects and were approved by the Health Research Ethics Committee of the Faculty of Medicine, Ciputra University (Indonesia) [No: 107/EC/KEPK-FKUC/III/2024].

Programs of high-intensity exercise

The intervention program was conducted on the athletics field of FIKK UNESA between 06:00 and 08:00 AM under the supervision of coaches from the Department of Sports Coaching Education, FIKK UNESA, to ensure that all participants performed the exercises correctly. The high-intensity exercise protocol consisted of sprint training at 85–95% HRmax, followed by interval running at 50–60% HRmax, repeated 10 times across 3–4 sets with a 3-minute rest between sets. This high-intensity exercise program was carried out over four weeks with a frequency of three sessions per week. Training intensity was monitored using the Polar Heart Rate Monitor H10.

Ice bath therapy procedure

The ice bath therapy procedure involved submerging the body up to the shoulders in a recovery ice bath tub filled with ice water at a temperature of 10–15°C for 5–15 minutes per session (Bleakley et al., 2012; Vaile et al., 2008; Banfi et al., 2008). Ice bath therapy was administered three times per week for four weeks in a controlled indoor environment. Each ice bath session was given 60 minutes after the high-intensity exercise intervention.

Data collection

Blood samples (4 cc) were drawn from the cubital vein at two time points: before (pre) and after (post) the four-week intervention. The collected blood samples were centrifuged for 15 minutes at 3000 rpm to separate the serum. Once separated, the serum was analyzed to determine the concentrations of IL-6, MDA, and CK activity. IL-6 concentration was measured using ELISA Kits (Cat. No.: E-EL-H6156; Human Interleukin 6 (IL-6) ELISA Kit; Elabscience Biotechnology Inc., USA). MDA levels were assessed using a Colorimetric Assay Kit (TBA Method) (Cat. No.: E-BC-K025-S; Malondialdehyde (MDA); Elabscience Biotechnology Inc., USA). CK activity was measured using an Activity Assay Kit (Cat. No.: E-BC-K558-S; Creatine Kinase (CK); Elabscience Biotechnology Inc., USA).

Statistical analysis

The data analysis began with a normality test using the Shapiro–Wilk test. Data with a normal distribution were further analyzed using parametric tests. A paired sample t-test was used to compare pre- and post-intervention conditions within each group, while an independent sample t-test was employed to assess differences between groups. The effect size was calculated using Cohen's d. Cohen classified effect sizes as small ($d = 0.2$), medium ($d = 0.5$), and large ($d \geq 0.8$) (Sullivan & Feinn, 2012). A significance level of $p < 0.05$ was used to determine statistically significant differences between groups.

Results

Observational data revealed no significant differences between groups in terms of age, anthropometric characteristics, or several physiological parameters (all $p > 0.05$) (Table 1). We detected a significant reduction in Interleukin-6 (IL-6), Malondialdehyde (MDA), and Creatine Kinase (CK) activity concentrations between pre- and post-treatment in the ice bath therapy (IBT) group (all $p \leq 0.005$) (Figures 1–3). Furthermore, we observed significant between-group differences in post-treatment IL-6, MDA, and CK activity levels, with the IBT group demonstrating markedly lower values compared to the control group (all $p \leq 0.05$) (Table 2).

Table 1. Descriptive characteristics of the study participants

Parameters	CTR; n=10	IBT; n=10	p-value
Age, yrs	21.40 ± 0.97	21.30 ± 1.06	0.828
Height, m	1.72 ± 0.04	1.72 ± 0.02	0.708
Weight, kg	64.20 ± 4.26	64.10 ± 2.56	0.950
Body mass index, kg/m²	21.81 ± 0.74	21.67 ± 0.74	0.665
Resting heart rate, bpm	64.20 ± 4.16	67.20 ± 6.72	0.245
Systole blood pressure, mmHg	117.40 ± 2.55	116.50 ± 2.27	0.415
Diastolic blood pressure, mmHg	75.30 ± 3.27	74.70 ± 3.62	0.702
Oxygen saturation, %	98.10 ± 1.10	97.70 ± 1.06	0.418
VO2max, mL/kg/min	47.13 ± 3.52	49.13 ± 4.69	0.296
Practice experience, yrs	11.60 ± 1.08	11.90 ± 1.73	0.647

CTR: Control group; IBT: Ice bath therapy. Data are presented as mean ± standard deviation (SDs). p-value was obtained using a independent sample t-test.

Figure 1. IL-6 (pg/mL) assessment pre and post treatment in each group. Data are presented as mean ± standard deviation (SDs). *Significant difference between pre and post intervention; p < 0.05. p-value was obtained using a paired sample t-test.

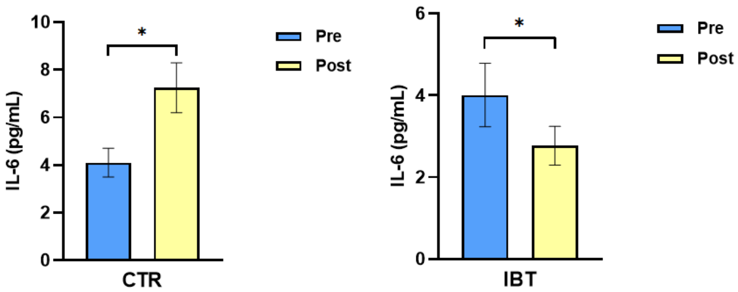

Figure 2. MDA (nmol/mL) assessment pre and post treatment in each group. Data are presented as mean ± standard deviation (SDs). *Significant difference between pre and post intervention; p < 0.05. p-value was obtained using a paired sample t-test.

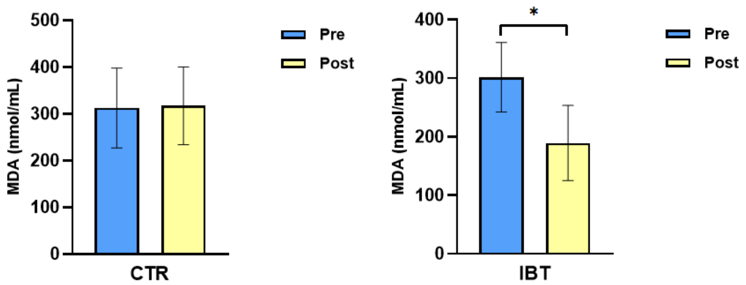

Figure 3. CK Activity (U/L) assessment pre and post treatment in each group. Data are presented as mean ± standard deviation (SDs). *Significant difference between pre and post intervention; p < 0.05. p-value was obtained using a paired sample t-test.

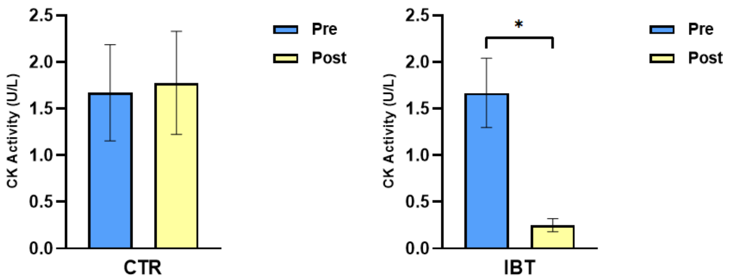

Table 2. The difference of IL-6, MDA concentrations and CK Activity between pre and post treatment between group

Variable	Group (n=10) CTR	IBT	P value	Effect size
	mean ± SDs	mean ± SDs		
Pre-IL-6 (pg/mL)	4.11 ± 0.61	4.01 ± 0.77	0.754	0.143
Pre-MDA (nmol/mL)	312.50 ± 85.65	301.50 ± 59.34	0.743	0.149
Pre-CK Activity (U/L)	1.67 ± 0.52	1.66 ± 0.37	0.993	0.022
Post-IL-6 (pg/mL)	7.25 ± 1.05	2.77 ± 0.47*	0.001	5.507
Post-MDA (nmol/mL)	317.25 ± 83.42	189.25 ± 64.09*	0.001	1.721
Post-CK Activity (U/L)	1.78 ± 0.55	0.25 ± 0.07*	0.001	3.902
Δ-IL-6 (pg/mL)	3.15 ± 1.39	-1.24 ± 0.85*	0.001	3.811
Δ-MDA (nmol/mL)	4.75 ± 45.82	-112.25 ± 34.63*	0.001	2.881
Δ-CK Activity (U/L)	0.11 ± 0.30	-1.42 ± 0.37*	0.001	4.542
Change-IL-6 (%)	80.88 ± 41.07	-29.49 ± 12.74	0.001	3.630
Change-MDA (%)	2.43 ± 14.77	-37.75 ± 10.74	0.001	3.111
Change-CK Activity (%)	8.01 ± 16.85	-84.45 ± 5.43	0.001	7.388

Delta (Δ)
*Significant at CTR $p < 0.05$
Effect size (ES) were evaluated using Cohen's d
p-value was obtained using a independent sample t-test
CTR: Control group; IBT: Ice bath therapy
Data are presented as mean ± standard deviation (SDs).

Discussion

This study aimed to examine the effects of ice bath therapy following high-intensity exercise on markers of inflammation and oxidative stress in adolescent males. The results demonstrated a significant reduction in interleukin-6 (IL-6), creatine kinase (CK), and malondialdehyde (MDA) levels after the application of ice compression, indicating its potential effectiveness as a recovery intervention. The large effect sizes observed—particularly in CK (d = 3.9) and IL-6 (d = 5.5)—may seem unusually high for a 4-week intervention. However, given the consistency of the training protocol, the specificity of the biomarkers, and the controlled administration of cryotherapy, these results are plausible. High-intensity repeated sprint protocols can induce substantial physiological stress, particularly in young athletes, making them highly responsive to recovery interventions in the short term. These findings are consistent with previous research highlighting the benefits of cryotherapy in reducing exercise-induced muscle damage and systemic inflammation (Hohenauer et al., 2015; Ihsan et al., 2021; Sánchez-Ureña et al., 2018). In adolescent populations, the immature antioxidant systems and fluctuating hormonal profiles may heighten post-exercise inflammatory responses. Cryotherapy, by reducing tissue temperature and blood flow, may mitigate these exaggerated responses more effectively than in adults. This aligns with the notion that adolescent athletes might experience more pronounced recovery benefits due to their heightened physiological reactivity. The observed reductions in these biomarkers support the role of cold-based therapy in modulating post-exercise inflammatory and oxidative responses, particularly in high-intensity training contexts. This reinforces the notion that the timely implementation of cryotherapeutic strategies can facilitate physiological recovery in adolescent athletes.

The reductions in IL-6, MDA, and CK activity may be attributed to several physiological mechanisms associated with ice compression. The application of cold induces localized vasoconstriction, limiting blood flow to damaged tissues and reducing the infiltration of inflammatory cells, thereby suppressing the production of pro-inflammatory cytokines such as IL-6 (Mawhinney, Jones, & Low, 2017). Moreover, the decrease in tissue temperature slows metabolic processes and enzymatic activity, reducing the generation of reactive oxygen species (ROS), which is reflected in lower MDA levels, a marker of lipid peroxidation (White & Wells, 2013). Ice compression also contributes to the preservation of muscle membrane integrity by reducing muscle cell permeability, thereby minimizing CK release into the bloodstream (Kusmierczyk et al., 2024). Collectively, these mechanisms illustrate how cold-based interventions promote physiological recovery following high-intensity exercise.

A notable decrease in IL-6 was observed following the application of ice compression, suggesting the downregulation of acute inflammatory processes. This result aligns with the findings of Jurecka et al. (2023), who demonstrated that cold interventions can suppress IL-6 expression following exhaustive exercise through cytokine modulation. Similarly, CK—a classic indicator of muscle membrane disruption—was significantly reduced in the post-treatment group, reinforcing the hypothesis that cryotherapy may protect muscle fiber structure after intense physical exertion. These findings are consistent with Kusmierczyk et al. (2024), who reported reduced CK activity in subjects undergoing cryotherapy before eccentric exercise. Furthermore, MDA levels, which reflect oxidative damage to cell membranes, were significantly lowered after intervention. This corroborates Siqueira et al. (2017), who showed that repeated cryotherapy exposure diminishes ROS production and mitigates oxidative stress following muscle injury. Overall, the biomarker profiles observed in this study suggest that ice compression exerts a multifaceted influence on recovery by simultaneously addressing inflammation, muscle damage, and oxidative stress.

Although efforts were made to standardize training and intervention conditions, several potential confounding factors could have influenced the results. These include individual differences in physical effort, hydration status, nutritional intake, and sleep quality—all of which may modulate inflammatory and oxidative stress markers. Future studies should consider controlling or monitoring these variables more systematically.

The outcomes of this study offer meaningful implications for sports science practitioners and recovery program designers, particularly for adolescent athletes involved in high-intensity exercise. As adolescents are in a critical phase of physiological development, they may be more susceptible to muscle damage and inflammatory responses compared to adults (Faigenbaum & Myer, 2010; Peake, 2019). Therefore, the implementation of ice compression—a cost-effective, accessible, and non-invasive modality—can be particularly valuable in youth athletic settings. The significant reductions in IL-6, CK, and MDA levels highlight the potential of this approach to not only accelerate recovery but also to prevent long-term physiological stress that could hinder training consistency and athletic progression. These findings support the integration of structured cryotherapeutic interventions into adolescent training programs and emphasize the importance of biomarker monitoring to assess recovery efficacy. From a practical perspective, the findings support the integration of ice bath therapy into regular recovery routines for adolescent athletes involved in high-intensity training. Given its accessibility and non-invasive nature, cryotherapy can serve as a valuable tool for coaches and sports practitioners aiming to minimize muscle damage and accelerate recovery. Long-term implementation, combined with individualized monitoring, could potentially enhance training consistency and reduce injury risk during developmental years.

Despite these promising results, several limitations should be acknowledged. This was a single-center study without participant or assessor blinding, which may introduce bias in intervention delivery or outcome interpretation. The relatively small sample size and exclusive focus on male adolescents limit the generalizability of the findings to broader populations, including females and adult athletes. Additionally, the study employed a short-term measurement protocol, with biomarkers assessed only before and shortly after the intervention. Future studies should consider longer follow-up periods to examine the extended effects of ice compression on performance and recovery outcomes. Furthermore, comparative investigations across various cryotherapy modalities—such as cold-water immersion, localized ice packs, and whole-body cryostimulation—are warranted to optimize recovery strategies, as each may elicit different physiological effects (Hohenauer et al., 2015). No participant dropouts or adverse effects were reported throughout the intervention period, indicating the feasibility and safety of the protocol for adolescent athletes.

Conclusions

This study demonstrated that ice bath therapy following high-intensity exercise significantly reduced levels of interleukin-6 (IL-6), malondialdehyde (MDA), and creatine kinase (CK) activity in adolescent males. These findings support the efficacy of cryotherapy in reducing inflammation, oxidative stress, and muscle damage, reinforcing its relevance as a non-invasive recovery strategy for young athletes. The results contribute novel evidence to the limited body of research on cryotherapy in adolescent populations, particularly using biochemical markers. Further research is warranted to determine the optimal duration, frequency, and water temperature of ice baths in this group, as well as to examine long-term safety and effectiveness. These findings may inform recovery protocols in school-based or amateur sports programs, where accessible and evidence-based interventions are essential for promoting safe and consistent training.

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Authors' and translators' details:

Adi Pranoto	adipranoto@unesa.ac.id	Author
Irmantara Subagio	irmantarasubagio@unesa.ac.id	Author
Gigih Siantoro	gigihsiantoro@unesa.ac.id	Author
Afif Rusdiawan	afifrusdiawan@unesa.ac.id	Author
Mochamad Purnomo	mochamadpurnomo@unesa.ac.id	Author
Donny Ardy Kusuma	donnykusuma@unesa.ac.id	Author
Bhekti Lestari	bhektilestari@unesa.ac.id	Author
Muhammad Kharis Fajar	muhammadfajar@unesa.ac.id	Author
I Dewa Made Aryananda Wijaya Kusuma	dewawijaya@unesa.ac.id	Author
Bekir Erhan Orhan	bekirerhanorhan@aydin.edu.tr	Author
Rahmatya Ikhwanurrosida	lingolinkpro@gmail.com	Translator