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Research Article

Impact of borage extract and 8-week aerobic training on liver autophagy genes Beclin-1 and Parkin in male Wistar rats with NAFLD

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Abstract

NAFLD is one of the chronic liver diseases closely related to metabolic disorders. Borage and physical activity improves oxidative indices. Also, Beclin-1 is a key gene in the autophagy process, and Parkin acts as a protective mechanism against liver damage. So, this study aims to investigate the effect of aerobic training and borage extract on the expression of Beclin-1 and Parkin genes in the liver cells in NAFLD. In this experimental study,40male Wistar rats weighing were divided into 4groups. (control, supplement, training), and training+supplement. The data were analyzed using SPSS 25 software. Training and borage extract had no significant effect on Beclin-1 expression (P=0.267), with fold changes of 1.05±0.07 for training, 1.07±0.06 for borage extract, and 1.12±0.08 for training+borage extract, compared to the control. However, Parkin expression increased significantly (P=0.006), with fold changes of 1.15±0.08 for Training, 1.18±0.09 for borage extract, and 1.20±0.10 for training+borage extract. Post hoc confirmed a significant increase in Parkin expression in the training+borage extract group compared to the control (P=0.035). This study showed that it is possible that the consumption of borage extract and aerobic training together and alone have an increasing the expression of Parkin. So, both borage extract and aerobic training have individual benefits for improving liver health by targeting Beclin-1 and Parkin pathways, and their combination could provide a more robust approach to managing NAFLD by promoting effective autophagy, mitophagy, and mitochondrial function. This dual intervention could reduce liver damage, inflammation, and fibrosis, ultimately improving metabolic outcomes in individuals suffering from NAFLD.

Key Words: **Aerobic training, Borage extract, Autophagy, Beclin-1, Parkin, NAFLD**

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Introduction

Non-alcoholic fatty liver disease is one of the chronic liver diseases closely related to metabolic disorders. Therefore, NAFLD positively correlates with obesity, insulin resistance, and type 2 diabetes (T2D) (Ok et al., 2018; Shewale et al., 2015). The global prevalence of NAFLD is 25%, with the highest prevalence in the Middle East and South America and the lowest in Africa (An et al., 2021). Therefore, lifestyle intervention, including dietary changes and regular exercise, is the cornerstone of managing patients with NAFLD (Booth et al., 2012; Mäkelä et al., 2022).

Also, due to its antioxidant properties, borage improves oxidative indices. Borage oil contains mainly (15–20%) gamma-linolenic acid (GLA), which reduces the lipid content and increases the oxidation of beta-fatty acids, showing this plant's liver-protective effect. In fact, GLA is quickly converted by the elangas enzyme. It is converted to DGLA, which is involved in oxidative metabolism by cyclooxygenase and lipoxygenase to produce anti-inflammatory eicosanoids. This product affects inflammation and pain (Ibrahim & Alshammaa, 2023; Khatri et al., 2012; Mustonen & Nieminen, 2023; Naghdi Badi et al., 2007; Urrestarazu et al., 2019).

Physical activity is also a first-line treatment and an important preventive measure for patients with NAFLD, but the underlying mechanisms are unclear. Physical activity significantly increases autophagy flux by restoring lysosomal function, including lysosomal proteolysis and maintaining lysosomal acidification, thereby contributing to increased autophagy clearance and the subsequent reduction of hepatic steatosis. Autophagy is a key cell protection mechanism to cope with various stress conditions (Chun et al., 2017; Keating et al., 2015). Therefore, based on studies, it can be concluded that combining borage extract with aerobic training may have synergistically enhance metabolic health, reduce inflammation, and improve mitochondrial function, leading to better outcomes in conditions like non-alcoholic fatty liver disease (NAFLD) compared to either intervention alone. This approach is novel

because it integrates the potential anti-inflammatory and antioxidant properties of borage extract with the established benefits of aerobic exercise. While both have shown individual benefits for metabolic health, their combined effects on liver function, mitochondrial efficiency, and overall metabolic balance have not been thoroughly explored, making this combination a promising area for further investigation.

In non-alcoholic fatty liver disease (NAFLD), Beclin-1 plays a key role in autophagy, helping to maintain cellular health by removing damaged organelles and proteins. Parkin is involved in mitophagy, a process that specifically targets damaged mitochondria for degradation and increases the accumulation of liver fat. Both proteins are critical for managing cellular stress and inflammation in NAFLD (Farzanegi & Abbaszadeh, 2022; Taji et al., 2017).

Interventions, such as lifestyle changes, medications, or specific signaling modulators, can influence Beclin-1 and Parkin activity by enhancing autophagy and mitophagy, potentially reducing liver damage, inflammation, and fibrosis. These interventions may promote improved mitochondrial function, mitigate oxidative stress, restore metabolic balance and helping to manage or reverse the progression of NAFLD (Ma et al., 2020; Undamatla et al., 2023; Wang et al., 2019; Williams & Ding, 2015; Williams et al., 2015; Zarringol, 2016). Therefore, this study aims to investigate the effect of 8 weeks of aerobic training and consumption of borage extract on the serum concentration of liver enzymes, fat profile, and Beclin-1 and Parkin genes in the liver cells of rats suffering from non-alcoholic fatty liver disease.

Materials and Methods

Animals

In this experimental study, 40 male Wistar rats aged 8 to 10 weeks weighing 250 to 300 grams were divided into 4 groups (N = 10). The groups were: control group (suffering from fatty liver and receiving enough water and food/without training and supplements); supplement group (suffering from fatty liver and receiving 200 mg of borage extract); training group (suffering from fatty liver and aerobic training, daily for 8 weeks); and training + supplement group (suffering from fatty liver and receiving 200 mg/kg borage supplement and performing aerobic training for 8 weeks). At the end, 0.5 cc of venous blood was taken from all animals to perform relevant tests, and then the Beclin-1 and Parkin genes were extracted and measured by the PCR method.

Borage extract

Borage extract (Borago officinalis): First, the desired plants were ground and passed through 18 to 35 meshes. Then, the desired amount was weighed and soaked with the relevant solvent for 2 hours. In the percolation method, we put a strainer at the end of the container, and then we transfer the plant that was soaked with solvent to the container. If needed, we add solvent so that it covers one centimeter above the plant's surface. After that, the decanter lid is placed so that the solvent and the plant mix for 24 hours. After 24 hours, open the decanter valve so the liquid extract comes out drop by drop. After extracting, we transfer them to the balloon for concentration and connect them to the rotary device. Depending on the amount of extracts and their concentration, the concentration process may take several hours to several days. Hydro alcoholic borage extract was prepared by the percolation method at the Research Institute of Plant and Pharmaceutical Sciences. The ratio of solvent to plant is 1.5. A 0.5 cc extract was fed to mice by the gavage method 5 days a week (Zamansoltani et al., 2008). The extract is typically derived from the seeds of the borage plant, as they are rich in bioactive compounds like GLA, alpha-linolenic acid (ALA), and other fatty acids.

Exercise training protocol

In the first week after the induction of non-alcoholic fatty liver, the rats entered the adaptation phase for the research project implementation, and they were introduced to aerobic training five days a week. Aerobic training in the acclimatization phase consists of 20 minutes of running on a treadmill with a zero incline at a speed of 15 meters per minute. The first 5 minutes are warm-ups, and the last 5 minutes are cool-downs.

In the first week, 30 minutes of running on the treadmill at a speed of 27 meters per minute and VO_{2max} 50–60% (aerobic training with moderate intensity) was performed; the first 5 minutes were for warming up, and the last 5 minutes were for cooling down (at a speed of 15 meters per minute). Gradually, during 8 weeks, the speed increased, and every week it increased by 1-2 m/min and reached 32 m/min in the eighth week (Table 1) (Hedayati et al., 2019).

Poisoning method

Table 1. The structured training protocol over an 8-week of the study

	Adaptability	Week							
		1	2	3	4	5	6	7	8
Set (repeat)	0	10	11	12	13	13	14	14	15
Intensity (meter / minutes)	10	14	16	18	20	22	24	26	28
Rest (minutes)	2	2	2	2	2	2	2	2	2

Description: This table presents the structured training protocol over an 8-week period, detailing the progression in sets, intensity (measured in meters per minute), and rest periods. The adaptability column represents the initial phase before the progressive increments begin.

Table 2. Macronutrient compositions of high-fat and standard diet (% of total energy intake)

Variables	standard diet(%)	high-fat diet (%)
Fat	12	60
Carbohydrate	57	30
Protein	28	10
Others	3	3

Conducting real-time tests

To perform the PCR reaction based on the Master Mix preparation instructions of Pars Tos Company, the reactions in PCR tubes with a final volume of 20 microliters included 10 microliters of master mix, one microliter of forward primer, one microliter of reverse primer, one microliter of cDNA, and seven microliters treated with DEPC. The reactions were prepared and run according to the type of genes in different temperature programs.

Statistical analysis

Data were analyzed using SPSS version 25 (IBM Corp., Armonk, NY, USA). The normality of the data distribution was assessed using the Shapiro-Wilk test. The one-way analysis of variance (ANOVA) was used to compare the mean of quantitative variables among study groups. Also, the Bonferroni post-hoc test was used for pairwise comparison between the groups, in case of significant main effect. For gene expression analysis, fold change values were calculated using the 2^(-ΔΔCt) method with β-actin as the housekeeping gene. The results are presented as mean, with a 95% confidence interval (CI). A significance level of $P<0.05$ was considered statistically significant. Data visualization was performed using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA) to illustrate group comparisons and expression changes.

Results

The difference in the expression of hepatic Beclin-1 and Parkin genes based on one-way ANOVA test is presented in Table 3. There was no significant difference in the expression of Beclin-1 gene in the liver among the study groups (P=0.239). As shown in Figure 1, Beclin-1 expression was the highest in the group with training + borage extract, increased by 1.10 ± 0.13 fold compared to the control group. Also, the fold change in the expression of this gene in the training and borage extract groups were 1.03 ± 0.06 and 1.04±0.04, respectively. However, eight weeks of aerobic training and borage extract have no significant effect on the hepatic Beclin-1 autophagy gene expression in male Wistar rats with non-alcoholic fatty liver disease.

Regarding to the expression of Parkin gene in the liver, a significant difference was observed among the study groups (P = 0.006). As presented in figure 2, a higher increase was obsesrved in the expression of hepatic Parking following borage extract supplementation (1.22 ± 0.09). However, the Bonferroni post-hoc test (Table 4) indicate that the change in this group was not statistically significant. In contrast, the increase in the hepatic autophagy gene expression Parkin in the training group (1.14 ± 0.05; P=0.005) and the extract-receiving + exercise group (1.18 ± 0.05; P=0.035) was significant compared to the control group. Therefore, eight weeks of aerobic training and borage extract significantly affecting the expression of the hepatic Parkin autophagy gene in male Wistar rats with non-alcoholic fatty liver disease.

Discussion

In this research, the effect of receiving the extract of borage extract alone or with aerobic exercise on the expression of liver autophagy genes such as Beclin-1 and Parkin in male rats with non-alcoholic fatty liver disease has been investigated. The results of this study showed that the consumption of borage extract alone or together with aerobic training can increase Beclin-1, but it was not statistically significant. In all three groups receiving borage extract, aerobic training, and borage extract + aerobic training, there was a statistically significant increase in the expression of the hepatic parkin gene.

In general, the results of previous studies indicate a relationship with non-alcoholic fatty liver disease and metabolic syndrome.

Table 3. One-way analysis of variance (ANOVA) test results for Beclin-1 and Parkin gene expression in the liver

Variables		Df	Average of squares	F	P	Effect size
	Corrected model	3	0.01	1.46	0.267	0.239
Beclin-1	Initial values	1	19.37	2895.36	<0.001	0.995
	Group	3	0.01	1.46	0.267	0.239
	Error	14	0.007			
	Corrected model	3	0.046	6.28	0.006	0.557
	Initial values	1	24.13	3302.61	<0.001	0.995
Parkin	Group	3	0.046	6.28	0.006	0.557
	Error	14	0.007			

Description: This table presents the results of a one-way ANOVA test analyzing the expression levels of Beclin-1 and Parkin genes in the liver. The table includes degrees of freedom (Df), mean squares, F-values, p-values, and effect sizes for each variable. The corrected model, initial values, group differences, and error terms are reported for both genes.

Table 4. Results of the post hoc Bonferroni test for the expression of the hepatic Parkin autophagy gene

Variable	Group	Control	Training	Borage extract	Borage extract + Training
Parkin	Control	-	0.005	0.234	0.035
	Training	-	-	0.736	0.999
	Borage extract	-	-	-	0.999
	Borage extract + Training	-	-	-	-

Description: This table presents the results of the Bonferroni post hoc test, comparing the expression levels of the hepatic Parkin autophagy gene among different experimental groups. The p-values indicate the statistical significance of pairwise comparisons between the Control, Training, Borage Extract, and Borage Extract + Training groups.

In studies of the effectiveness of exercise training with different intensities and durations on non-alcoholic fatty liver markers, physical activity is the first line of treatment and a non-alcoholic fatty liver preventive agent. Aerobic exercise reduces inflammation by regulating the AMPK and PPAR protein expression level and ultimately improves liver steatosis and inflammation through the AMPK-PPAR signaling pathway. In meta-analysis studies, it was found that aerobic and resistance exercise both improve NAFLD. Also, physical activity increases the autophagic flow by restoring the function of the lysosome and thus reducing liver steatosis. Autophagy is a supportive cellular mechanism to face stressful conditions (Gunadi et al., 2020).

The beclin-1 gene plays an important role in the cell death pathway and is a key molecule in controlling The activity of this gene is regulated by several mechanisms, including post-translational modification, protein-protein interaction, and intracellular localization. The role of parkin in an alcoholic fatty liver is characterized by a supportive mechanism for mitochondrial damage. Mitophagy works to maintain mitochondrial function. Reduction of mitophagy through parkin reduces mitochondrial respiration capacity, increases liver fat accumulation, and increases insulin resistance. On the other hand, many medicinal plants with different doses of anti-inflammatory properties have been used in studies, such as bora-

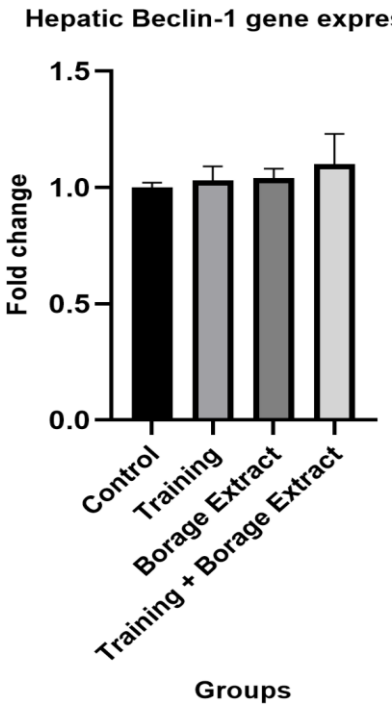

Figure 1. Hepatic Beclin-1 gene expression changes (fold change) in the studied groups.

Description: This bar plot illustrates the fold change in Beclin-1 gene expression in the liver across four experimental groups: Control, Training, Borage Extract, and Training + Borage Extract. Data are presented as mean ± standard deviation (SD). No significant differences were observed between the groups, indicating that neither training nor borage extract significantly altered hepatic Beclin-1 expression levels.

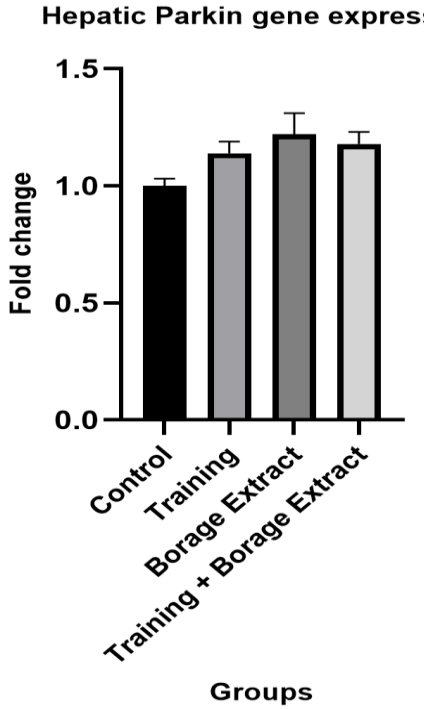

Figure 2. Hepatic Parkin gene expression changes (fold change) in the studied groups.

Description: This bar plot displays the fold change in Parkin gene expression in the liver across four experimental groups: Control, Training, Borage Extract, and Training + Borage Extract. Data are presented as mean ± standard deviation (SD). The results indicate an increasing trend in Parkin expression in the intervention groups compared to the control group, suggesting a potential effect of training and borage extract on hepatic Parkin autophagy gene expression.

-ge extract. The GLA present in borage extract reduces apoptosis by reducing the expression of Bax-Bcl2. GLA activates autophagy and autophagosome fusion. Lysosomes, the LKB1-AMPK-MTOR signaling pathway, positive regulation of mRNA, and Beclin -1 expression help (Gunadi et al., 2020; Liang et al., 2021). In addition, there are very few studies regarding the effects of borage extract and aerobic exercise on the expression of Beclin-1 and Parkin genes in patients with NAFLD, described below.

Therefore, in a study conducted in 2020 by Gunadi et al. on the effect of different exercise intensities on autophagy and its relation to fat metabolism in the liver of Wistar rats, it was found that the expression of beclin.ATG5.LC3 genes compared to the control group increased. The studies showed that autophagy increases due to physical exercise in patients with non-alcoholic fatty liver (Gunadi et al., 2020). Also, based on the study of Lemus-Conejo et al., it was observed that the decrease in the expression of Beclin-1 in mice with a high-fat diet causes oxidative conditions, fat accumulation, and a lack of homeostasis in liver cells (Lemus-Conejo et al., 2020; Lemus-Conejo et al., 2022).

In a study conducted in 2021 by Yaxu Liang et al. on the effect of gamma-linolenic acid on preventing fat metabolism disorder in 12 mouse liver cells under the effect of palmitic acid by balancing autophagy and apoptosis through the LKB1-AMPK-Mtor pathway, It was proven that excess fat accumulation is seen in non-alcoholic fatty liver disease, while GLA, as a polyunsaturated fatty acid, plays a role in reducing this deposition. This study investigated the effectiveness and regulatory mechanism of GLA (Mm 100) on the fat metabolism of liver alpha cells subjected to palmitic acid (Mm 400). GLA helps to reduce fat content, increase fat beta-oxidation, decrease triglyceride and cholesterol levels, and increase mRNA and protein expression of CPT1 and PPAR. Also, GLA helps to improve oxidative stress, positive regulation of mRNA levels of superoxide dismutase and glutathione peroxidase, and reduction of reactive oxygen species (ROS). GLA helps to reduce apoptosis by reducing the expression of BAX-BCL2 and apoptosis. GLA activates autophagy, autophagosome-lysosome fusion, the LKB1-AMPK-Mtor signaling pathway, positive regulation of mRNA, expression of Beclin-1 proteins, and autophagy-related LKB-1. In fact, GLA, as a nutritional supplement in the prevention and treatment of non-alcoholic fatty liver disease, is involved in regulating lipid metabolism and autophagy (Liang et al., 2021).

The setting of mitophagy generated by mitochondrial injury is the most appropriate way to characterize Parkin (DP, 2010; Narendra et al., 2008). Parkin synthesizes poly-ubiquitin chains on proteins in the outer mitochondrial membrane as part of Parkin-mediated mitophagy signaling (Undamatla et al., 2023).

According to certain research, Parkin loss speeds up the development of important NAFLD disease characteristics, and loss of mitophagy happens early in the pathogenesis of the illness. Furthermore, diminished mitophagy was not only a concomitant characteristic of a fatty liver; rather, the absence of Parkin-mediated mitophagy hindered mitochondrial respiration ability and intensified the buildup of liver fat as well as the degree of insulin resistance following high-fat diet feeding (Edmunds et al., 2020). Generally, more data about the hepatic Parkin gene and how it functions in non-alcoholic fatty liver disease is not known. So, the effect of borage extract and aerobic training on Beclin-1 and Parkin in liver tissue in the context of NAFLD is intriguing and could offer new insights into potential therapeutic strategies for the disease.

Borage extract is rich in GLA, a polyunsaturated fatty acid known for its anti-inflammatory and antioxidant properties. It has been shown to modulate cellular stress pathways and promote mitochondrial health, potentially enhancing autophagy and mitophagy. In the context of Beclin-1, borage extract may boost the autophagy process, facilitating the removal of damaged cellular components, reducing liver cell damage, and preventing the accumulation of dysfunctional proteins and organelles. For Parkin, borage's anti-inflammatory effects could indirectly enhance mitophagy by reducing oxidative stress and preserving mitochondrial function. This could potentially mitigate the accumulation of damaged mitochondria that is often seen in NAFLD, leading to improved cellular health and liver function (Chen et al., 2024; Liang et al., 2021).

Aerobic exercise is well-documented for its ability to improve mitochondrial biogenesis, enhance cellular metabolism, and reduce liver fat accumulation. Aerobic training has been shown to increase Parkin activity, promoting mitophagy to remove damaged mitochondria, thus reducing mitochondrial dysfunction, a hallmark of NAFLD. Regular aerobic exercise also enhances overall autophagic processes, including the activity of Beclin-1, which may contribute to better cellular maintenance and reduce the burden of misfolded proteins and damaged organelles in liver cells. Aerobic training may also improve insulin sensitivity, which could influence the activity of both Beclin-1 and Parkin, contributing to a reduction in liver inflammation and fibrosis (Chen et al., 2024; Ghareghani et al., 2018; Rosa, 2016).

Therfore, when combined, borage extract and aerobic training could have a synergistic effect on Beclin-1 and Parkin activity in NAFLD. Borage extract may enhance the autophagic and mitophagic processes, while aerobic training increases mitochondrial biogenesis and strengthens these pathways through consistent physical stress. Together, they could foster a more efficient system for clearing damaged cellular components, reducing oxidative stress, and promoting mitochondrial turnover. This combined effect could result in a reduction in liver

health in individuals with NAFLD. Furthermore, the antioxidant properties of borage extract could complement the benefits of aerobic exercise, potentially enhancing the recovery of liver cells from the oxidative damage associated with both metabolic dysfunction and physical activity. So, the results of the present study showed that consumption of borage extract and aerobic training for 8 weeks did not statistically change the amount of Beclin-1 gene but caused a significant increase in the amount of Parkin gene. Therefore, based on the study's findings, the hypothesis of the consumption of borage extract and aerobic training on improving the expression index of autophagy genes is accepted just for Parkin.

Conclusion

In conclusion, according to the previous studies and the current studies, it is stated that physical activity of any kind, especially aerobic, can reduce oxidative stress, inflammation, and apoptosis, which can improve the severity of the disease in people with NAFLD. Also, the combined effect of aerobic training and borage extract can probably be more effective on the oxidative status, apoptosis of liver cells, and liver autophagy genes (in the present study, an increase in Parkin gene expression was observed after 8 weeks of intervention) and improve or treat NAFLD disease.

What is already known on this subject?

Non-alcoholic fatty liver disease is one of the chronic liver diseases closely related to metabolic disorders.

What this study adds?

Aerobic exercise can reduce oxidative stress, inflammation, and apoptosis, which can improve the severity of the disease in people with NAFLD.

Organ Cross-Talk Tips:

- Crosstalk involving autophagy (Beclin 1) and mitophagy (Parkin) further supports liver function by mitigating oxidative damage and inflammation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study has the code of ethics from the Ethics Committee of Islamic Azad University, Tehran, Iran (ethical code: IR.IAU.SRB.REC.1401.194).

Informed consent Animal study

Author contributions

Conceptualization: A.B., H.A.N., M.Gh, F.Gh.; Methodology: A.B., H.A.N., M.Gh, F.Gh.; Software: H.A.N.; Validation: A.B. Formal analysis: M.Gh.; Investigation: F.Gh.; Resources: A.B., H.A.N., M.Gh, F.Gh.; Data curation: A.B., H.A.N.; Writing - original draft: A.B.; Writing – review & editing: M.Gh.; Visualization: F.Gh.; Supervision: H.A.N. Project administration: H.A.N.; Funding acquisition: H.A.N

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