Resveratrol Mitigates Acrylonitrile-induced Thyroid and Adrenal Toxicity in Rats

Samer I. Sabeeh [1*], Ahmed Q. Al-Awadi [1]

Abstract

Background: Acrylonitrile (AN) poses significant health risks as a potent multi-site carcinogen, causing toxicity in various organs including the brain, lung, liver, kidney, stomach, and adrenal glands. AN-induced oxidative stress and lipid peroxidation lead to DNA damage and organ dysfunction, including adrenal necrosis and haemorrhages. Resveratrol, a potent antioxidant, offers potential protection against acrylonitrile-induced damage. Method: This study utilized 55 adult male Sprague-Dawley rats divided into four groups: control, Resveratrol-treated, AN-treated, and AN + Resveratrol-treated. Animals were subjected to oral administration of AN or Resveratrol for 90 days. Serum levels of thyroid hormones (T3, T4, TSH) and adrenaline were measured using ELISA, while histopathological changes in thyroid and adrenal glands were examined microscopically. Results: AN exposure significantly decreased serum concentrations of T3, T4, TSH, and adrenaline, accompanied by histopathological alterations including necrotic follicles and adrenal congestion. Resveratrol treatment mitigated these effects, restoring hormone levels and preserving tissue architecture. Conclusion: Resveratrol exhibits protective effects against AN-induced toxicity by enhancing thyroid and adrenal hormone production and attenuating histopathological changes in thyroid and adrenal glands. Its antioxidant properties mitigate oxidative stress and maintain cellular homeostasis, suggesting its potential therapeutic utility in combating AN-related health hazards. Further research is warranted to elucidate the mechanisms underlying Resveratrol's protective effects on adrenal vascular function.

Keywords: Acrylonitrile, Resveratrol, Thyroid, Adrenal, Toxicity

Significance | Resveratrol counters acrylonitrile's adverse effects, preserving thyroid and adrenal function, evidenced hormonally and histologically, suggesting therapeutic potential.

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Introduction

Acrylonitrile (AN) is a multi-site tumorigen and displays noteworthy toxicity, as evidenced by occupational epidemiology and animal studies (Bates et al., 2023). AN injures multiple organs, including the brain, lung, liver, kidney, stomach, and adrenal gland (Albertini et al., 2023; Humadi et al., 2020). The reaction formula for acrylonitrile is CH3H3N, and it is a very toxic chemical used in making acrylic fiber and plastic solvents. The chemical structure of acrylonitrile shows a strong triple bond that contributes to its high polarity (Humadi et al., 2020).

Acrylonitrile is a colorless and white or yellow opaque liquid due to the cyano groups (CNEO) in its units, which provide strong polarity and increase the softening points of AN (Al-Azzawi and Tamimi, 2008). Sometimes referred to as "vinyl cyanide," acrylonitrile is an organic chemical commonly used in industry as a monomer for manufacturing resins and polymers. The two principal pathways of exposure are inhaling AN through its evaporation into the air or drinking polluted water contaminated from xenobiotic sources, as

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it is well-soluble and relatively stable in water. Additionally, contaminated drinking water and cigarette smoke may provide exposure to AN (Al-Azzawi and Yaseen, 2016).

AN can also be released in minor amounts through the combustion of plant matter (Kobets et al., 2022). It produced adrenal necrosis in 40-55% of the animals studied (Szabo and Sandor, 1997). AN triggers the depletion of cellular sulfhydryls, such as glutathione (GSH), which likely exacerbates oxidative stress (Puppel et al., 2015).

Both reagents react with glutathione (GSH), together depleting cellular GSH concentrations. Additionally, thiocyanate, produced from cyanide released by acrylonitrile (AN), serves as a substrate for peroxidases (e.g., myeloperoxidase, lactoperoxidase), generating hypothiocyanite. This compound can also deplete levels of reduced GSH and is formed from natural sources such as supplements, deoxycholic acid, acetoacetate imosing sulfoxidation, or N-chlorination. In tissues with peroxidase activity and hypothiocyanite formation, AN and CNEO may act additively to weaken cellular defenses against oxidative damage (EPA IRIS, 2011).

Acrylonitrile induces oxidative stress and lipid peroxidation, forming DNA-reactive intermediates like reactive oxygen species (ROS) and malondialdehyde, which damage genomic material (Arlandson et al., 2001; EPA, 2011). It causes adrenal necroses and hemorrhages at doses as low as 200 mg/kg body weight. AN is produced from acrylamide using immobilized Brevibacterium CH1 cells with nitrile hydratase activity (Humadi et al., 2020). Decreases in GSH concentrations due to oxidative stress are associated with overproduction of ROS, likely generated by acrylamide (AA). Excess ROS production is known to oxidize proteins and generate lipid peroxidation (LPO) products and markers of oxidative injury (Ramadhan and Khudair, 2018).

Acetonitrile is a main byproduct of acrylonitrile production, though it accounts for only 2-4% of the yield, leading to strict restrictions on acrylonitrile production (Yuan et al., 2019). Thiocyanate exposure can result in goiter by blocking iodine uptake by the sodium-iodine symporter in the thyroid (Wolff, 1998; Tonacchera et al., 2004; De Groef et al., 2006). It also inhibits thyroid hormone production, potentially leading to thyroid autoimmunity (Patani et al., 2023). Oxidative stress, resulting from dysregulation of adrenal hormone secretion, is a significant health issue in humans (Patani et al., 2023).

Resveratrol (trans-3,4-trihydroxystilbene; RES) is a plant-derived stilbenoid polyphenolic product found in a wide variety of plants, including grapes, peanuts, and blueberries. It is particularly abundant in grapes and their products, including red wine, which contains relatively high amounts of resveratrol (Abdulla and Al-Okaily, 2022). Resveratrol is classified as a stilbenoid, a type of naturally occurring phenolic compound commonly used as an antioxidant in the medical field and for treating various diseases (Abdulla and Al-Okaily, 2022).

Resveratrol is employed as a chemopreventive agent (Rieder et al., 2012), an antioxidant, and an anti-inflammatory agent (Khayoon and Al-Rekabi, 2020; Alghetaa et al., 2018). It also acts as an immunomodulatory agent, capable of directly modulating the immune response and preventing the production of cytokine storms (Alghetaa et al., 2021). Additionally, resveratrol is used as an anti-diabetic agent (Khudair and Al-Okaily, 2022).

Materials and Methods

Chemicals

Acrylonitrile solution (≥99%) (Sigma–Aldrich GmbH, Steinheim, Germany) was administered via gastric gavage (PO) for 90 days at a dose of 40 mg/kg body weight per day (ICRP, 2002). The test solution was prepared the day before the experiments by diluting 0.8 ml of AN in 100 ml of distilled water (0.8% v/v). Resveratrol (200 mg) (Now Company, Utah City, USA) was orally administered via gastric gavage (PO) for 90 days at a dose of 20 mg/kg body weight (Chang et al., 2012). The doses were freshly prepared shortly before administration.

Experimental Animals

Fifty-five adult male Sprague Dawley rats, weighing an average of 260±10 g, were obtained from the Breeding Unit of the Higher Institute for the Diagnosis of Infertility and Assisted Reproduction Techniques at Al-Nahrain University. They were housed in plastic cages at the College of Veterinary Medicine, University of Baghdad, under controlled conditions (temperature: 25±3°C, relative humidity: 50±5%). Before starting the experimentation, the rats were acclimatized to laboratory conditions for one week. They were maintained under a 12-hour light/12-hour dark cycle with free access to pellets and tap water.

Experimental Protocol

The rats were divided into four groups as follows:

Group I (n=10): Negative control.

Group II (Resveratrol group) (n=15): Rats received a single dose of Resveratrol (20 mg/kg) orally for 90 days.

Group III (ACN group) (n=15): Positive control group receiving a single daily dose of AN (40 mg/kg) orally for 90 days.

Group IV (ACN + Resveratrol group) (n=15): Rats were given water containing a single dose of AN (40 mg/kg) and water containing Resveratrol (20 mg/kg) for 90 days.

Following administration (day 90), blood samples were taken to measure serum triiodothyronine (T3), thyroxine (T4), thyroid-stimulating hormone (TSH), and adrenaline hormones. The rats were euthanized by intraperitoneal administration of ketamine (90 mg/kg body weight) and xylazine (40 mg/kg body weight) to obtain thyroid and adrenal glands.

Hormonal Assay

The serum levels of thyroid hormones (triiodothyronine-T3, tetraiodothyronine-T4) and epinephrine were measured by ELISA (Elabscience MyBioSource, India).

Ethics Approval

The trial was conducted with the approval of the Ethics Committee of Animal Care, University of Baghdad, College of Veterinary Medicine.

Microscopical Examination

For histological analysis of the thyroid and adrenal glands, tissue samples were immediately fixed in 10% neutral buffered formalin for 24 hours, processed into routine paraffin blocks, sectioned (4-6 µm), and stained with hematoxylin and eosin. Photographs were taken using a 17-megapixel microscopic camera. Light microscopy was utilized (Bancroft and Gamble, 2007).

Statistical Analysis

The data were entered into IBM SPSS version 26.0 for statistical analysis. The mean and standard deviation (SD) of continuous variables were calculated, and differences between groups were analyzed using the analysis of variance (ANOVA) test, followed by the LSD test. Statistically significant differences were declared if $P \leq 0.05$.

Result

Hormonal Assay

The results indicated a significant decrease ($P<0.05$) in serum concentrations of T3, T4, TSH, and adrenaline hormones in the AN-treated group compared with both the control and Resveratrol-treated groups (Figure 1). The Resveratrol-treated group showed a significant increase in these hormones compared with the control group, except for the adrenaline hormone, which was mildly decreased. The AN + Resveratrol group showed significant improvement in serum T3, T4, TSH, and adrenaline hormone values, although they were still lower than the controls (Figure 3).

Table 1: Effect of Acrylonitrile, Resveratrol, and their combination on serum T3, T4, TSH, and Adrenaline concentrations in adult male rats (Mean±SD).

Histopathology

The thyroid gland in the Resveratrol group showed normal architecture, comprising thyroid follicles of different shapes and sizes with a single layer of thyrocytes and parafollicular cells (C-cells) (Figure 2). The adrenal gland also exhibited normal architecture (Figure 3). Other sections showed blood vessel medulla congestion (Figure 3), perivascular congestion with mild necrosis in secretion cells (Figure 5). In the AN-treated group, the thyroid gland histopathology revealed continuous use of AN for 90 days resulted in thyroid follicles of irregular shapes and sizes with unclear lining of thyrocytes and parafollicular cells (Figure 6).

Histopathological findings included necrotic thyroid follicles (Figure 6a) and MNCs infiltration in the follicular lumen (Figure 6b). Additionally, interstitial hemorrhage was observed (Figure 7). In the adrenal gland, there was severe congestion of blood vessels (Figure 8) and focal necrosis between the cortex (zona reticularis) and the medulla (Figure 8). Furthermore, mild infiltration of MNCs was noted between the medullary nets (Figure 9).

Discussion

The present results showed a significant change in the serum levels of T3, T4, and TSH in control and adult male rats treated with acrylonitrile (ACN). The reduction in the level of thyroxin hormone observed in this study may be due to iodine deprivation, leading to the thyroid gland's failure to synthesize thyroxin, resulting in hypothyroidism (Kaneko et al., 1997). Alternatively, this reduction may occur secondary to pituitary insufficiency, as anoxia and acrylamide can induce hypothyroidism and hyperlipidemia by increasing the rate of thyroid hormone (T3) elimination from circulation through elevated biliary excretion, caused by plasma protein binding disorders (Arrak, 2010).

The present results align with Alwan et al. (2016), where acrylonitrile administered at a dose of 5 mg/kg BW/day for 45 days resulted in a significant decrease in serum T3, T4, and TSH. The mechanism by which thyroid hormone levels are reduced in the ACN group may be similar to the mechanism used by lead acetate and ACN, involving the inhibition of hypothalamic peptide thyroid-releasing hormone (TRH) or thyrotropin-stimulating hormone (TSH). Additionally, the oxidative properties of ACN, which release free radicals, contribute to the decrement of thyroid hormones, especially T3. ACN-induced cytotoxicity raises levels of lipid peroxidation, depressing the level of antioxidant enzymes and increasing cellular oxidative stress. This also reduces equivalents such as glutathione (GSH), indicating that essential thiol (-SH) groups are depressed.

The high reduction potential of thiols allows them to reduce oxidizing chemicals involved in harmful free-radical reactions. This mechanism involves multiple modes of action, including scavenging, direct disruption of molecular cross-links, restoration of antioxidant enzymes, and accelerated regeneration of their cofactors (Sardi, 2015). The resulting global reduction in thiol levels might lead to the disruption of 5-D enzyme configurations, inhibiting the formation of T3 from T4, as the 5-D configuration represents a reduced state of the enzyme (Arrak, 2010).

A light microscopic study of thyroid tissue in animals affected by acrylonitrile (AN) revealed significant histological changes due to enhanced cellular oxidative stress and depressed antioxidant levels (Zhao et al., 2019). The histological alterations included marked deterioration and necrosis of the thyroid follicles, evidenced by vacuolation of the epithelial lining. This deterioration is likely due to the oxidative stress induced by AN, leading to oxidative damage of biological macromolecules. AN increases the production of

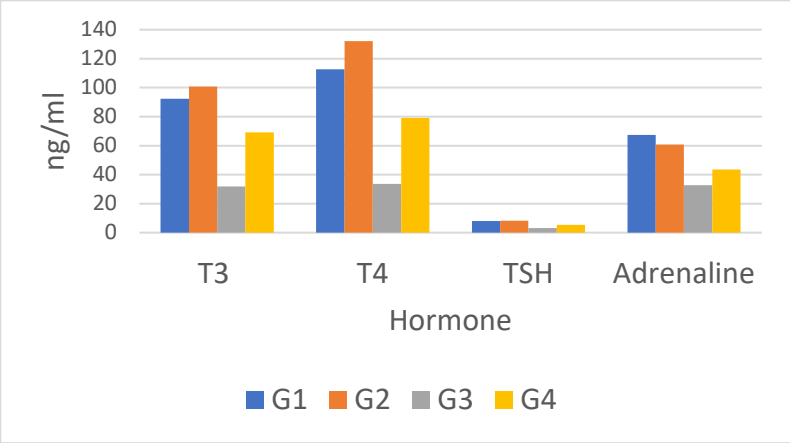


Figure 1. Effect of resveratrol, Acrylonitrile, and /or their combination on T3, T4, TSH and adrenaline in adult male rats.

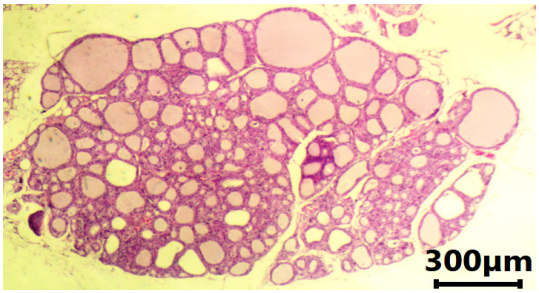


Figure 2. Section of thyroid gland from resveratrol group shows normal architecture (Hand E).

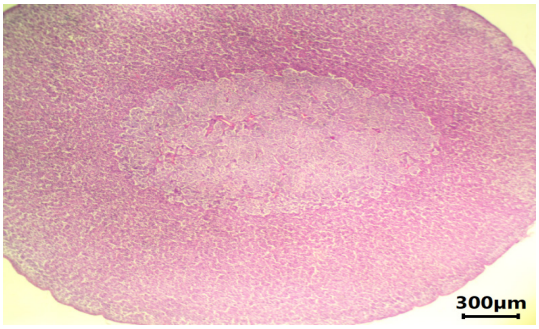


Figure 3. Section of adrenal gland from resveratrol group shows normal architecture (Hand E).

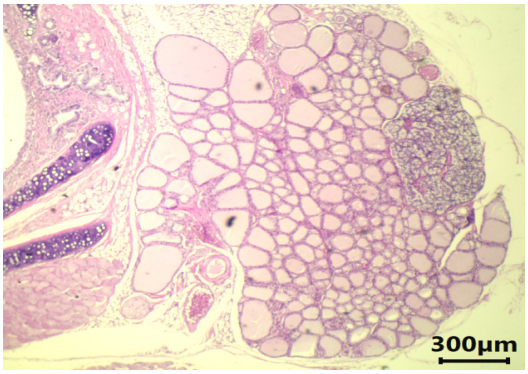
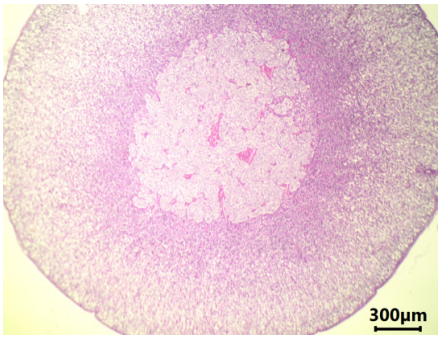


Figure 4. Section of thyroid and parathyroid glands from Acrl 40% treated with Resveratrol shows normal histological



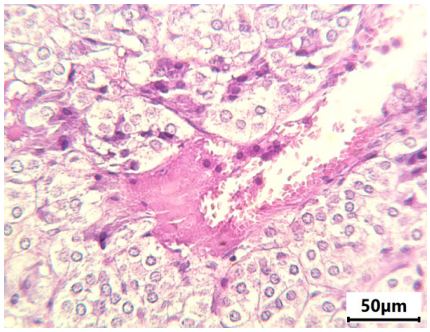


Figure 5. Section of adrenal gland from ACN +Resveratrol group

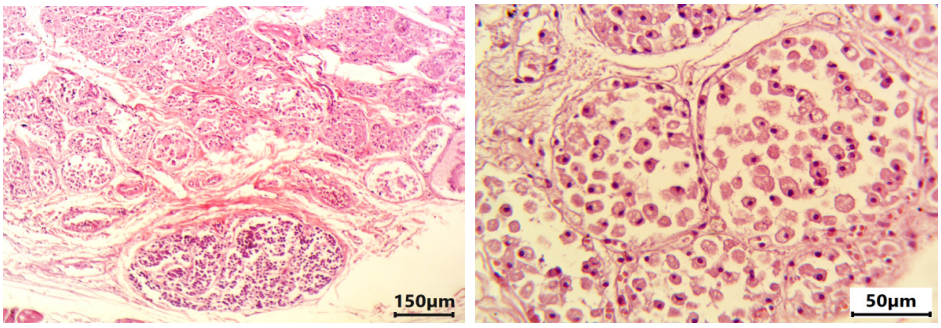


Figure 6. Section of thyroid gland from Acrl 40% group shows (a) shows severe necrosis of thyroid follicles and necrosis of parathyroid gland, (b) shows loss of follicles epithelia and infiltration of macrophages in the follicle's lumen. (H and E).

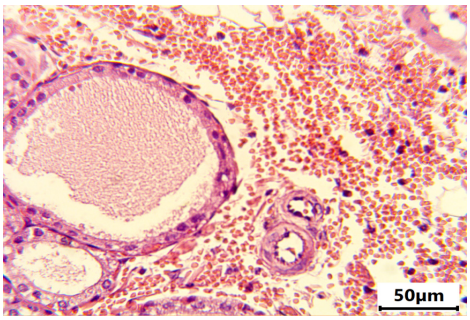


Figure 7. Section of thyroid gland from Acrl 40% group shows interstitial hemorrhage (Hand E).

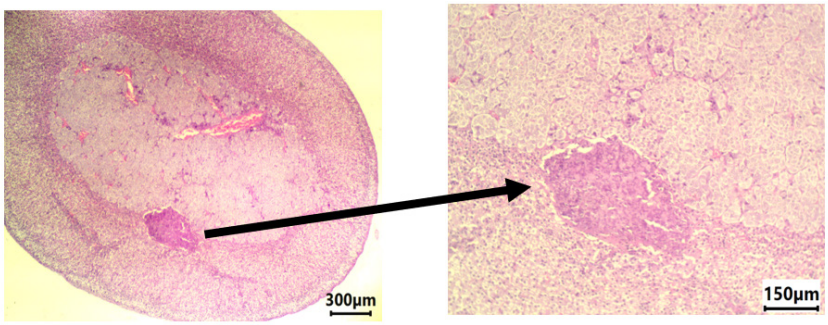


Figure 8. Section of adrenal gland from Acrl 40% group shows severe congestion of blood vessels and focal necrotic area between the cortex (zona reticularis) and the medulla (arrow). (Hand E).

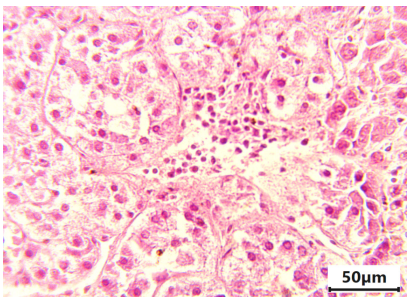


Figure 9. Section of adrenal gland (medulla) from Acrl 40% group shows mild infiltration of MNCs between the medullary nests. (Hand E).

reactive oxygen species (ROS), such as superoxide ions, hydroxyl radicals, and hydrogen peroxide, which cause lipid peroxidation, DNA damage, membrane damage, alteration of gene expression, and apoptosis (Dang et al., 2018). Additionally, the decline in TSH, which is responsible for the normal morphological appearance of thyroid follicles, further exacerbates these changes (Ibrahima, 2018).

AN also induces adrenal pathology in animals (Verma and Rana, 2009). In the ACN + Resveratrol (Res) group, Res acts as an antioxidant, mitigating the toxic effects of AN and protecting cells and tissues from the destructive effects of ROS and other free radicals (Fig 1). At the cellular level depicted in Fig 6, degeneration and necrosis of the thyroid follicles were attenuated with Res administration (Fig 6A, Fig 6B, Fig 6C, Fig 6D). Resveratrol alleviates endotoxemia-associated adrenal insufficiency by suppressing oxidative/nitrative stress and protecting cells against oxidative stress-induced injury. It is well known that resveratrol exhibits potent antioxidant activities by quenching ROS (Chandra et al., 2007) and increasing the activity of antioxidant enzymes such as catalase and glutathione peroxidase (Floreani et al., 2003).

Numerous studies have indicated the role of resveratrol against oxidative stress in various tissues, such as the lung (Zhang et al., 2014), kidney (Moridi et al., 2015), and heart (Gutiérrez-Pérez et al., 2011). In the present study, resveratrol treatment decreased levels of malondialdehyde (MDA) in adrenal glands while increasing total antioxidant capacity (T-AOC) and the activities of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) in adrenals obtained from LPS-treated mice (Tab 3). Resveratrol significantly protected mice against endotoxemia-associated adrenocortical hyporesponsiveness (Table 1). These results suggest that the protective effect of resveratrol against endotoxemia-associated adrenocortical hyporesponsiveness is at least partly due to its potent antioxidant properties. Future research should explore the various mechanisms underlying the protective effect of resveratrol on adrenal vascular function.

Conclusion

The present study demonstrated that acrylonitrile (AN) exposure significantly impacts thyroid and adrenal gland function in adult male rats by inducing oxidative stress and depressing antioxidant levels. Histopathological analysis revealed marked deterioration and necrosis of thyroid follicles and severe adrenal congestion. AN exposure also resulted in significant reductions in serum levels of thyroid hormones (T3, T4, TSH) and adrenaline. These findings are consistent with previous studies indicating that AN disrupts endocrine function through oxidative damage and alterations in hormone synthesis pathways.

Resveratrol (Res) administration ameliorated these effects, demonstrating its potent antioxidant properties. Resveratrol treatment not only improved the serum hormone levels but also mitigated the histopathological damage induced by AN. The protective effects of Resveratrol were evident through the decreased malondialdehyde (MDA) levels and increased activities of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) in the adrenal glands.

These results suggest that Resveratrol can serve as a therapeutic agent to counteract the toxic effects of AN, highlighting its potential in oxidative stress-related endocrine disruptions. Further research is necessary to explore the detailed mechanisms by which Resveratrol exerts its protective effects and to assess its potential applications in clinical settings.

similarities in demographic parameters seen in several research highlight the consistency and significance of our findings within the wider context of evaluating male infertility.

Author contributions

S.I.S., A.Q.A.A. conducted the experiments, performed the statistical analysis, wrote, edited, and reviewed the article.

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Competing financial interests

The authors have no conflict of interest.

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