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Original Article

Anti-Fibrotic and Anti-Oxidant Potential of Aminoguanidine in Hypothyroidism-Associated Cardiovascular and Renal Injury in Male Rat, Biochemical and His Ological Ev Dence

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Abstract

Introduction: This study aimed to evaluate the therapeutic potential of aminoguanidine (AG), an inducible nitric oxide synthase (iNOS) inhibitor, in attenuating renal and cardiac fibrosis and oxidative stress in juvenile rats with experimentally induced hypothyroidism.

Methods: Hypothyroidism was induced by administering 0.05% propylthiouracil (PTU) in drinking water for six weeks. Thirty-five rats were assigned to five groups: control, hypothyroid only, and three hypothyroid groups treated with AG (10, 20, or 30 mg/kg, i.p.). Serum thyroxine (T4), blood urea nitrogen (BUN), and creatinine levels were measured to assess thyroid and renal function. Oxidative stress markers including malondialdehyde (MDA), thiol content, superoxide dismutase (SOD), and catalase (CAT) were evaluated in serum, and aorta, heart, and kidney tissues. Cardiac and renal fibrosis was examined using Masson's trichrome staining.

Results: Rats receiving PTU alone developed overt hypothyroxinemia, as evidenced by a marked reduction in serum T4, together with elevated levels of BUN and creatinine. Cardiac and renal tissues from PTU-treated animals exhibited fibrosis, accompanied by increased malondialdehyde concentrations and concomitant decreases in antioxidant defenses, including thiol groups, superoxide dismutase, and catalase activity. Co-administration of AG effectively reversed these alterations, restoring serum T4 and tissue antioxidant parameters while lowering BUN, creatinine, and malondialdehyde levels. The highest dose of AG also decreased cardiorenal fibrosis compared to the PTU group.

Conclusion: The present findings demonstrate that pharmacological inhibition of iNOS attenuates oxidative stress and cardiorenal fibrosis in experimental hypothyroidism. Although aminoguanidine exhibits recognized limitations for clinical application due to safety concerns, these results support the involvement of the iNOS pathway in hypothyroidism-associated tissue injury and highlight iNOS modulation as a potential mechanistic target for future investigations.

Keywords: Hypothyroidism, Aminoguanidine, Nitric oxide synthase, Oxidative stress, Fibrosis

Introduction

Hypothyroidism is a condition that disrupts multiple metabolic pathways, affecting not only thyroid hormone levels but also the function of vital organs such as the heart and kidneys.[1,2] This endocrine disorder is often linked to oxidative stress a state where the overproduction of free radicals overwhelms the antioxidant defense mechanisms, leading to lipid peroxidation and tissue damage. Such oxidative imbalance has been increasingly recognized as a central contributor to cardiac and renal complications observed in hypothyroidism.[3]

Hypothyroidism impairs cardiovascular function by reducing heart rate, increasing vascular resistance, and elevating cholesterol levels, which may contribute to

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arterial narrowing.[4] Therefore, hypothyroidism leads to cardiovascular disorders, and in the absence of thyroid hormones, morphological and structural changes occur in cardiac cells, which lead to changes in cardiac hemodynamics.[5] Hypothyroidism also indirectly affects renal blood flow and glomerular filtration by affecting the cardiovascular system.[6] Hypothyroidism is also suggested to have a role in the pathophysiology of renal diseases.[7]

A growing body of evidence suggests that hypothyroidism contributes to fibrotic remodeling in vital organs such as the heart and kidneys, primarily through mechanisms involving oxidative stress and inflammatory signaling.[8-10] One of the key mediators in this process is inducible nitric oxide synthase (iNOS), [11] which becomes upregulated under hypothyroid conditions and promotes excessive nitric oxide production,[12] leading to nitrosative stress and tissue injury.[13] Aminoguanidine (AG), a selective iNOS inhibitor, has shown promise in attenuating these pathological changes by reducing oxidative damage and modulating fibrotic pathways.[14] In experimental models, AG has been associated with decreased collagen deposition and improved histological architecture in organs affected by endocrine dysfunction.[15,16]

It is suggested that iNOS contributes to inflammation by producing nitric oxide in response to pathological stimuli.[17] Excess activation of iNOS can exacerbate oxidative stress, thereby intensifying organ injury.[18] In this context, AG, a well-characterized experimental iNOS inhibitor, is employed in the present study as a pharmacological tool to probe the mechanistic contribution of iNOS-derived nitric oxide to hypothyroidism-associated cardiovascular and renal injury. Rather than being proposed as a definitive therapeutic agent, AG is used here to experimentally modulate iNOS activity and to explore how attenuation of iNOS-mediated nitric oxide overproduction may influence oxidative/antioxidative balance and tissue damage in this preclinical model.[1,19] Prior research has hinted at the capacity of AG to modulate oxidative stress markers, which makes it a compelling candidate for addressing the biochemical disturbances in hypothyroidism.[16] Given the interplay between thyroid hormone deficiency, oxidative imbalance, and fibrosis, pharmacological inhibition of iNOS with aminoguanidine in this experimental model provides mechanistic insight into how iNOS-derived nitric oxide may contribute to structural damage in the heart and kidneys during hypothyroid states, without implying any therapeutic applicability of AG.

Although previous studies have examined the antioxidant and anti-fibrotic properties of AG in various pathological contexts, its protective effects specifically against hypothyroidism-induced cardiorenal injury remain insufficiently explored.

Moreover, no prior research has comprehensively evaluated whether AG can simultaneously modulate oxidative stress markers, improve biochemical profiles, and attenuate fibrosis in both cardiac and renal tissues within a hypothyroid model. This gap in the literature underscores the need for a more integrated assessment of the mechanistic involvement of the iNOS pathway in endocrine-related organ injury, rather than suggesting any therapeutic applicability of AG.

Therefore, the unique contribution of the present study lies in providing the first combined biochemical, oxidative, and histopathological evaluation of AG in a PTU induced hypothyroidism model, with a specific focus on its ability to mitigate cardiorenal oxidative imbalance and tissue fibrosis. By addressing this gap, the study offers new mechanistic insight into the involvement of the iNOS pathway in hypothyroidism-associated cardiac and renal injury, without implying any therapeutic applicability of AG.

Herein, the current study aims to provide insights into whether AG can mitigate the oxidative imbalance and improve biochemical profiles and cardiorenal tissue fibrosis, thereby offering a potential therapeutic avenue to protect cardiac and renal tissues in the setting of hypothyroidism.

Materials and Methods

Animal groups

This investigation was conducted using thirty-five juveniles male Wistar rats with body weights ranging from approximately 55 to 60 grams, at the animal facility of Mashhad University of Medical Sciences. Laboratory rats were kept under standardized conditions, including regulated temperature and light cycles, with unrestricted access to food and water.

Male Wistar rats were allocated into five distinct groups (n = 7 per group): (1) a control group, (2) a Hypothyroid-only group, and three combinatory groups receiving PTU along with AG at daily dosages of 10, 20, or 30 mg/kg (Hypothyroid-AG 10, Hypothyroid -AG 20, and Hypothyroid -AG 30, respectively). Hypothyroidism was pharmacologically induced via the incorporation of 0.05% PTU into the drinking water .[9,10] For groups three through five, intraperitoneal injections of AG were administered daily, while equivalent volumes of saline were delivered to groups one and two. This treatment regimen was maintained for duration of six weeks (Table 1).

The selected AG doses (10, 20, and 30 mg/kg) were based on previously published studies demonstrating that AG exerts antioxidant, anti-inflammatory, and anti-fibrotic effects within this dosing range in rodent models without inducing toxicity. AG at 10 mg/kg has been shown to reduce oxidative stress and inflammation in diabetic rats .[20] The 20

Table 1. Studied groups and treatment with propyl thiouracil (PTU) or aminoguanidine (AG)

Group	Treatment	PTU in Drinking Water	AG Injection
1	Control	No	Saline
2	Hypothyroid	Yes (0.05%)	Saline
3	Hypothyroid-AG 10	Yes (0.05%)	AG 10 mg/kg
4	Hypothyroid AG 20	Yes (0.05%)	AG 20 mg/kg
5	Hypothyroid -AG 30	Yes (0.05%)	AG 30 mg/kg
6 Weeks			

mg/kg dose has demonstrated biochemical and antioxidant protection in liver injury models .[16,21] Moreover, 30 mg/kg has been widely used as an effective and safe dose in preventing oxidative and fibrotic organ damage .[16,22] Given this evidence, the graded dosing strategy in the present study was designed to evaluate potential dose dependent protective effects against hypothyroidism induced cardiorenal injury while remaining within established safety margins.

Upon completion of the treatment period, the rats were anesthetized using urethane at a dose of 1.4 g/kg, [15] after which blood specimens were obtained for the quantification of serum BUN, creatinine, and thyroxine levels. Subsequently, aorta, hearts and kidneys were excised following a rinse in chilled saline, weighed, and processed to create a 10% (w/v) tissue homogenate in a 1.15% potassium chloride solution.[16] After centrifuging the homogenized sample, the supernatant was collected to measure oxidative stress biomarkers, including malondialdehyde (MDA), thiol groups, superoxide dismutase (SOD) and catalase (CAT). A piece of heart and kidney was collected to be used for histological experiments.

Measurement of thyroxine, creatinine and BUN in serum

Serum concentrations of thyroxine, creatinine, and BUN were quantified using commercial method in Medical Laboratory.

Analysis of oxidative and antioxidative biochemical profiles in the serum, aorta, heart and kidney tissues

Levels of MDA, CAT, SOD, and thiol groups were determined in the serum, heart, aorta and kidney tissues using the protocols delineated in our previous studies.[23]

Histopathological evaluation of heart and kidney tissues

Heart and kidney tissues were excised and fixed in 10% neutral-buffered formalin for 72 hours. Following fixation, the samples were processed through standard paraffin embedding and sectioned at a thickness of 5 µm. The sections were subsequently deparaffinized in xylene, rehydrated through a graded ethanol series, and rinsed in 0.01 M phosphate-buffered saline (PBS, pH 7.4). Masson's trichrome staining was performed according to the manufacturer's instructions to evaluate collagen deposition and fibrosis. The stained sections were examined under a light microscope for histopathological assessment of fibrotic changes.[9,10,16,19]

Statistical Procedures

Data were shown as mean ± SEM and analyzed using ANOVA with Tukey-Kramer post-test; significance was set at $P < 0.05$.

Results

The effect of AG on the serum levels of thyroxine, creatinine and BUN

Administration of PTU resulted in a hypothyroidism status which was confirmed by a significant reduction in thyroxine levels in the serum when compared to the control group ($P < 0.001$). Hypothyroidism was accompanied with a marked elevation of serum BUN and creatinine concentrations when compared to the control group ($P < 0.001$, Figure 1). Relative to the hypothyroid group, animals receiving AG exhibited a statistically significant normalization of BUN levels across two higher tested doses. Additionally, creatinine concentration was notably reduced in the groups treated with the highest AG dose, while serum thyroxine level showed a statistically significant increment in all tested concentrations of AG ($P < 0.01$ to $P < 0.001$, Figure 1). Despite treatment with AG,

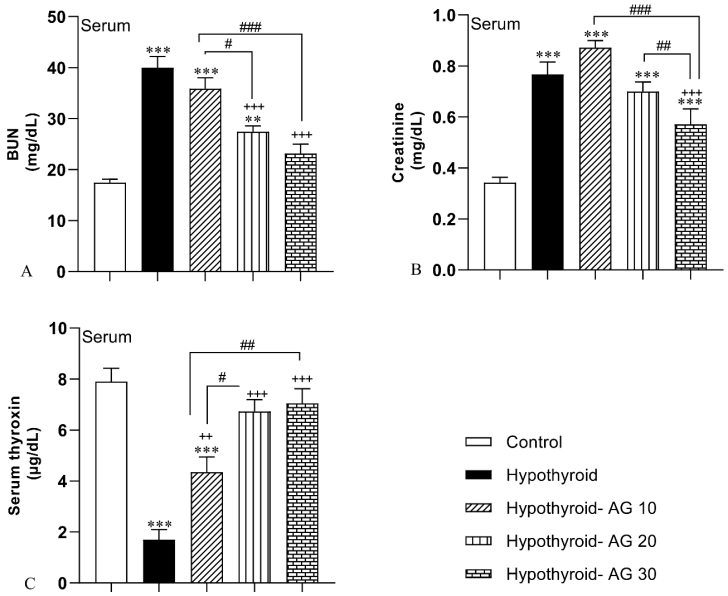


Figure 1. Serum level of BUN (A), creatinine (B) and thyroxine (C) in control animals, hypothyroid group (PTU), and PTU groups treated with AG (PTU+AG), (n= 7). Data are presented as mean ± SEM values. ** $P < 0.01$ and *** $P < 0.001$ compared to control group. ++ $P < 0.01$ and +++ $P < 0.001$ compared to group PTU. # $P < 0.05$, ## $P < 0.01$ and ### $P < 0.001$ compared of three concentrations of AG. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey-Kramer's post-test

creatinine concentration in hypothyroid animals remained significantly altered across all administered doses when compared to the control group ($P < 0.001$). Furthermore, thyroxine level in the cohort receiving the lowest AG concentration, and BUN levels in the cohorts receiving the low and medium AG concentrations also demonstrated a statistically significant deviation from control values ($P < 0.01$ to $P < 0.001$, Figure 1).

Administration of AG at its two highest dosages produced a significantly greater improvement in BUN and thyroxine levels compared to the lowest dose ($P < 0.05$ to $P < 0.001$, Figures 1A and 1C). Moreover, the highest AG concentration elicited a more pronounced reduction in creatinine levels relative to the low dose group ($P < 0.001$; Figures 1B). A further statistically significant difference in creatinine concentration was observed between the high and intermediate AG doses ($P < 0.01$; Figure 1B).

The effect of AG on the MDA level in the serum and aorta, heart and kidney tissues

MDA concentration in the serum, aorta, heart and kidney tissues were markedly elevated in animals administered PTU relative to the control group ($P < 0.001$, Figure 2). Treatment with AG at its two highest concentrations resulted in a significant attenuation of MDA levels in the serum, aorta, and kidney tissues when compared to the PTU group ($P < 0.01$ to $P < 0.001$, Figure 2). Additionally, MDA levels were notably elevated in the group receiving the highest AG dose relative to the PTU group ($P < 0.001$, Figure 2).

However, administration of AG at lower concentrations did not fully restore serum MDA level to those observed in the control group ($P < 0.05$ to $P < 0.001$, Figure 2A). Specifically, heart and aorta MDA levels in animals receiving the all doses of AG and kidney MDA level in the lowest dose group remained significantly elevated compared to control ($P < 0.01$ to $P < 0.001$, Figure 2). Furthermore, the reduction in serum, aorta and kidney MDA levels achieved by the higher AG concentrations was significantly greater than that observed with the lowest dose ($P < 0.05$ to $P < 0.001$, Figure 2). The highest AG concentration also caused a greater reduction of MDA levels in the serum and aorta compared to the medium dose ($P < 0.05$ to $P < 0.01$, Figure 2). Notably, pairwise comparisons revealed significant differences in heart MDA levels between high and medium AG doses, as well as between low and high doses ($P < 0.05$ to $P < 0.01$, Figure 2B).

The effect of AG on the thiol, SOD and CAT levels in the serum, aorta, heart, and kidney tissues

In animals treated PTU, a marked reduction in thiol, SOD, and CAT was observed in the serum, aorta, heart and kidney tissues compared to the control group ($P < 0.001$, Figures 3, 4 and 5). However, administration of AG at medium and high concentrations significantly restored thiol levels in the serum, aorta and heart tissues, SOD activity in the serum and kidney tissue, as well as CAT activity in the serum, aorta and heart tissues. Additionally, thiol level and CAT activity in the kidney tissue, SOD activity in the heart and aorta tissues were notably elevated in the group receiving the highest AG dose relative to the hypothyroid group ($P < 0.05$ to $P < 0.001$, Figures 3, 4 and 5).

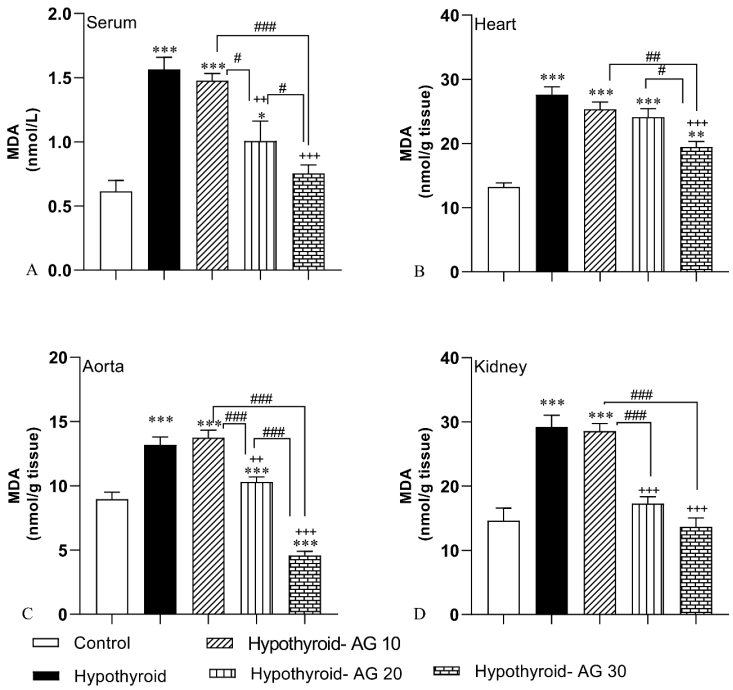


Figure 2. MDA concentration in serum (A), heart (B), aorta (C) and kidney (D) tissues in control animals, hypothyroid group (PTU), and PTU groups treated with AG (PTU+AG), (n= 7). Data are presented as mean ± SEM values
*, **, *** indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$ vs. control group
+, ++, +++ indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$ vs. PTU group
#, ##, ### indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$ among the three AG concentrations
Statistical analyses were performed using one-way ANOVA followed by Tukey–Kramer post hoc test

Despite these improvements, thiol and SOD levels in the heart and kidney tissues and CAT levels in serum, aorta, heart and kidney tissues of AG-treated PTU animals across all AG concentrations remained significantly lower than those of the control group. Similarly, thiol level in the serum, along with SOD activity in the aorta tissue of animals treated with the two lower AG doses, did not reach control values ($P < 0.05$ to $P < 0.001$), (Figures 3, 4 and 5). Specifically, aorta thiol level and serum SOD activity in animals receiving the lowest dose of AG remained significantly reduced compared to control ($P < 0.001$, Figure 2).

Comparative analysis revealed that the two higher AG concentrations produced significantly greater increases in serum and aorta thiol levels and serum SOD activity, as well as serum, aorta and heart CAT levels than the lowest dose ($P < 0.05$ to $P < 0.001$, Figures 3, 4 and 5). Furthermore, a statistically significant difference in the heart and aorta thiol levels and the aorta and kidney CAT levels were detected between the high and medium AG doses ($P < 0.05$ to $P < 0.01$, Figures 3 and 5). The high AG concentration also resulted in a significantly greater enhancement of kidney CAT and thiol levels, and aorta and kidney SOD activity compared to the low dose ($P < 0.05$ to $P < 0.001$, Figure 3, 4 and 5).

The effect of AG on the fibrosis in the heart and kidney tissues

The results of staining with Masson's trichrome revealed that hypothyroidism was associated with substantial fibrosis in the heart and kidney tissues compared to the control group ($P < 0.001$, Figures 6 and 7). In addition, our results showed that treatment by the highest dose of AG decreased fibrosis in the heart and kidney tissues compared to the hypothyroid group ($P < 0.001$, Figures 6 and 7). However, administration of AG at the highest concentration did not fully restore fibrosis change to those observed in the control group ($P < 0.01$ to $P < 0.001$, Figures 6 and 7).

Discussion

This study assessed how AG influences biochemical and histological changes caused by hypothyroidism, focusing on thyroid hormones, kidney function, heart and kidney fibrosis and oxidative stress in the serum, aorta, heart and kidney tissues. Aadministration of PTU via drinking water is a well-established method for inducing hypothyroidism.[24,25] Consistent with this protocol, the present study incorporated PTU into the drinking water to elicit a hypothyroid state in the experimental subjects.

The PTU-induced hypothyroidism model is widely used due to its reproducibility and its ability to mimic key biochemical and physiological features of human hypothyroidism, including reduced thyroid hormone levels, oxidative stress, and cardiorenal dysfunction.[26] PTU inhibits thyroid peroxidase and peripheral T4-to-T3 conversion, mechanisms that parallel the hormonal disturbances observed in patients. However, this model does not fully replicate autoimmune aetiologies such as Hashimoto's thyroiditis, nor does it reflect the typically gradual onset of hypothyroidism in humans.[27] These differences should be considered when extrapolating findings to clinical conditions.

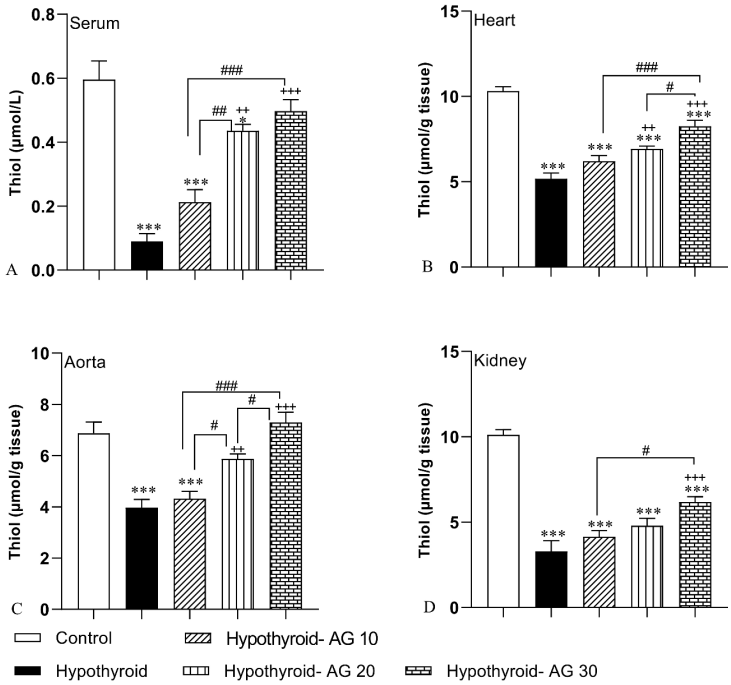


Figure 3. Thiol concentration in serum (A), heart (B), aorta (C) and kidney (D) tissues in control animals, hypothyroid group (PTU), and PTU groups treated with AG (PTU+AG), (n= 7). Data are presented as mean ± SEM values
*, *** indicate $P < 0.05$ and $P < 0.001$ vs. control group
++, +++ indicate $P < 0.05$ and $P < 0.001$ vs. PTU group
#, ##, ### indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$ among the three AG concentrations
Statistical analyses were performed using one-way ANOVA with Tukey–Kramer post hoc test

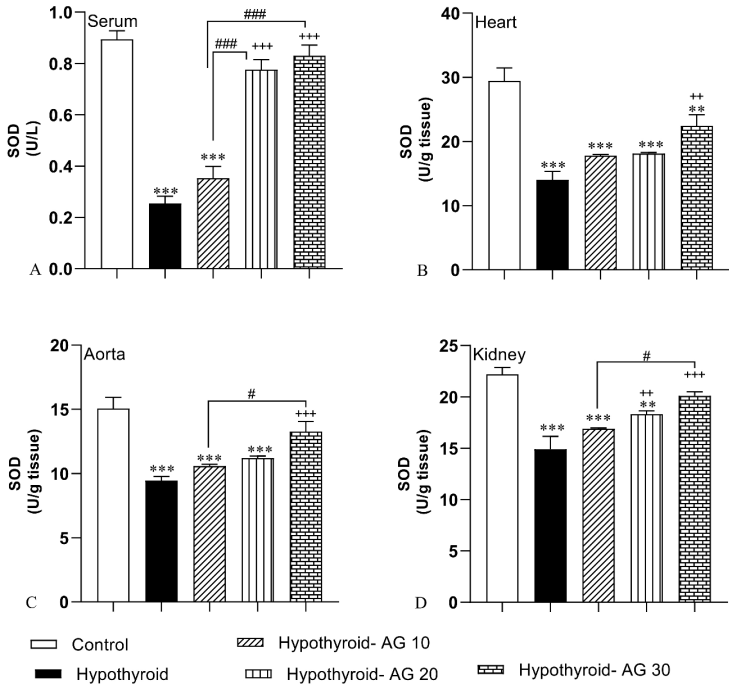


Figure 4. SOD level in serum (A), heart (B), aorta (C) and kidney (D) tissues in control animals, hypothyroid group (PTU), and PTU groups treated with AG (PTU+AG), (n= 7). Data are presented as mean ± SEM values
, * indicate $P < 0.01$ and $P < 0.001$ vs. control group
++, +++ indicate $P < 0.05$ and $P < 0.001$ vs. PTU group
#, ### indicate $P < 0.05$ and $P < 0.001$ among the three AG concentrations
Statistical analyses were performed using one-way ANOVA with Tukey–Kramer post hoc test

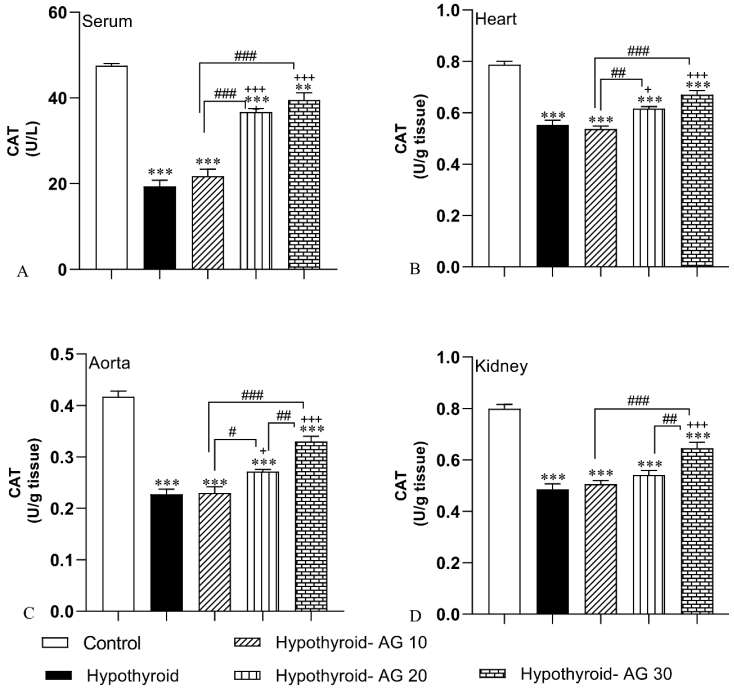


Figure 5. CAT level in serum (A), heart (B), aorta (C) and kidney (D) tissues in control animals, hypothyroid group (PTU), and PTU groups treated with AG (PTU+AG), (n= 7). Data are presented as mean ± SEM values
*** indicates $P < 0.001$ vs. control group
+, +++ indicate $P < 0.05$ and $P < 0.001$ vs. PTU group
#, ##, ### indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$ among the three AG concentrations
Statistical analyses were performed using one-way ANOVA followed by Tukey–Kramer post hoc test

Hypothyroidism was confirmed with a lower level of thyroxin in the serum of hypothyroid group compared to the control group which was followed by elevated levels of BUN and creatinine, indicating kidney dysfunction. In confirming with the current results, administration of thyroid hormone was reported to improve renal function in patients suffering from chronic kidney disease and subclinical hypothyroidism.[28]

In the same context, hypothyroidism is shown to be associated with a reduction in renal plasma flow and

glomerular filtration rate which might be responsible for elevated levels of BUN and creatinine which was seen in the current research.[29]

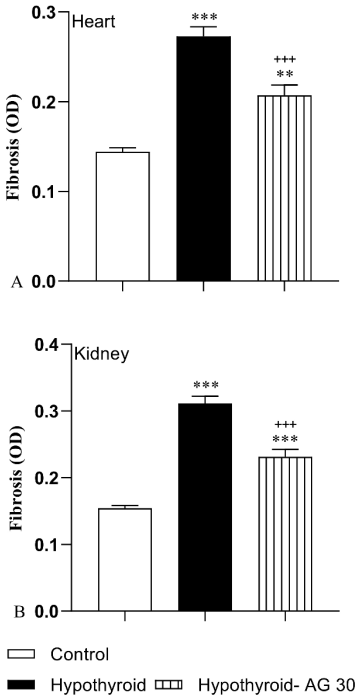


Figure 6. Fibrosis changes in heart tissue (A) and kidney (B) in control animals, hypothyroid group (PTU), and PTU groups treated with the highest dose of AG, (n= 7). Data are presented as mean ± SEM values. ** $P < 0.01$, and *** $P < 0.001$ compared to control group. +++ $P < 0.01$ compared to group PTU. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey-Kr mer's post-test

The results of current study also showed that hypothyroidism was accompanied with an oxidative stress status in the renal and cardiovascular system which confirmed by an increased level of MDA and a decrease in thiol, SOD and CAT in the kidney, serum, heart, and aorta. The present study demonstrated that hypothyroidism was associated with marked fibrotic changes in both cardiac and renal tissues, as confirmed by Masson's trichrome staining. These findings are consistent with previous studies reporting that hypothyroidism can promote extracellular matrix accumulation and collagen deposition, leading to fibrosis in multiple organs.[30] Considering these results it seems that oxidative stress has a main role in renal and cardiovascular dysfunction directly [3,31-33] or through induction of fibrosis in hypothyroidism conditions.[8,34]

Over activity of iNOS and over production of nitric oxide has been suggested to have a role in cardiovascular and renal diseases especially in hypothyroidism conditions.[35-37] In the present study iNOS inhibitor AG attenuated BUN and creatinine which confirms its improving effects on renal function in hypothyroid rats. In consistent with the current results, AG was previously reported to improve renal function in conditions such as sepsis, radiotherapy, and ischemia induced renal injury.[38-40]

While all concentrations showed some degree of improvement in the current research, the two higher concentrations of AG were more effective in lowering BUN and creatinine levels as well as in mitigating the rise in MDA concentration in heart and kidney tissues. MDA is commonly utilized as a biomarker of lipid peroxidation and

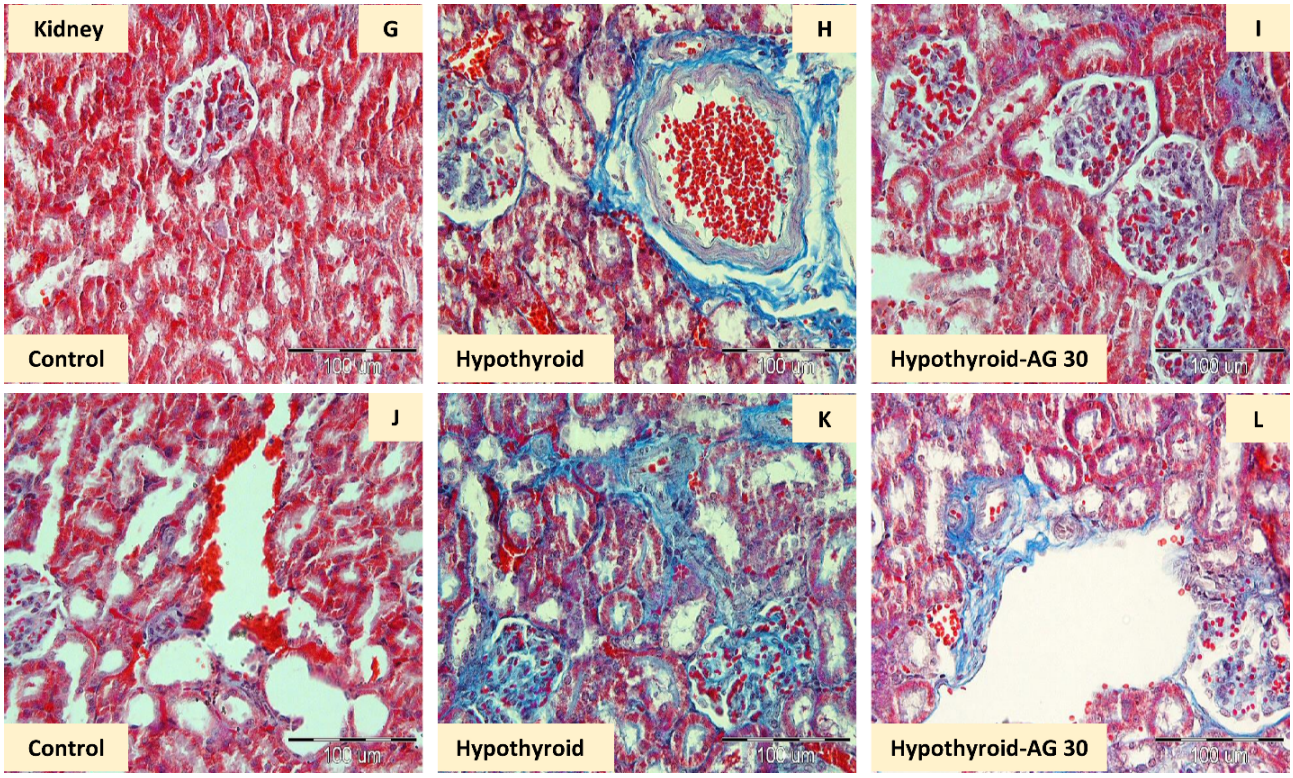


Figure 7. Histopathological evaluation by Masson's trichrome staining
Representative photomicrographs of heart (A-F) and kidney (G-L) tissues show normal collagen distribution in controls and increased collagen deposition in hypothyroid groups. Blue staining indicates collagen fibers, reflecting fibrosis. Administration of highest dose of AG reduced cardiac and renal fibrosis rendered by hypothyroidism (Scale bar = 100 µm)

oxidative stress;[41] therefore, its reduction by AG indicates potent free radical scavenging and antioxidant properties. In addition to reducing oxidative damage, AG treatment enhanced key antioxidant defense mechanisms. Administration of elevated doses of AG resulted in a marked restoration of SOD and CAT enzymatic functions, alongside a significant recovery in thiol group content within serum and cardiac and renal tissues. This restoration is particularly important because antioxidant enzymes are central to the neutralization of reactive oxygen species, thereby protecting cellular structures from oxidative insults.[42] Restoration of these enzymes, in parallel with the amelioration of MDA levels, reinforces the notion that AG exerts its protective effects not merely by directly scavenging free radicals, but also by upregulating the endogenous antioxidant defense systems.

Interestingly, the highest dose of AG attenuated renal and cardiac tissue fibrosis in the current research. Considering these results it seems the anti-fibrotic effects of AG is at least in part responsible for its renal and cardiovascular properties which were seen in the present study.

Transforming growth factor-β (TGF-β) has a critical role in collagen deposition and development the fibrosis. Interestingly, THs via inhibiting TGF-β signaling attenuate fibrotic responses.[43,44] Therefore, over activity of TGF-β signaling pathways in hypothyroidism seems to be followed by fibrosis and structural and functional changes leading to cardiovascular and renal dysfunction.[9,10] The reduction in collagen deposition observed in the AG-treated groups may be attributed to its antioxidant and anti-inflammatory properties, which have been shown to inhibit fibroblast activation and limit tissue remodeling.[12,15]

However, it should be emphasized that the involvement of the TGF-β pathway in the present study is a theoretical interpretation based on previously published evidence, as no molecular analyses (such as Western blotting or qPCR) were performed to directly measure TGF-β expression or downstream signaling components. Therefore, this mechanistic explanation remains speculative and should be interpreted with caution.

This represents a limitation of the current study, and future investigations incorporating molecular biology techniques are needed to confirm whether AG modulates TGF-β signaling in hypothyroidism-induced cardiac and renal fibrosis.

In addition, the translational applicability of the present findings is inherently limited. AG was used solely as an experimental iNOS inhibitor, and the results should not be extrapolated to clinical use. Furthermore, the PTU-induced hypothyroidism model does not fully replicate the complexity of human hypothyroidism, including its chronic progression, hormonal fluctuations, and comorbid metabolic or autoimmune features. These factors restrict the direct translation of the current results to human disease, underscoring the need for future studies employing clinically relevant models and evaluating safe, viable iNOS-modulating strategies.

However, despite the significant improvement, AG administration at the highest tested dose did not completely reverse fibrotic alterations to the levels observed in the control group, indicating that while AG mitigates fibrosis; its restorative effect may be partial or dose-dependent. The findings suggest that excessive iNOS-derived nitric oxide may contribute to hypothyroidism-induced fibrosis and cardiorenal injury. Pharmacological inhibition of iNOS with aminoguanidine in this experimental model provides mechanistic insight into the involvement of the iNOS pathway in these pathological changes. These results highlight the potential role of iNOS signaling in hypothyroidism-associated tissue injury, without implying any therapeutic development or clinical applicability of AG.

Another important finding was the differential improvement in thyroxine levels. AG was able to significantly increase thyroxine levels, suggesting that the underlying mechanism might require a robust antioxidant or metabolic modulation sufficient to correct the inhibitory effects of PTU on thyroid hormone synthesis. This observation highlights the complexity of the interaction between thyroid function and oxidative stress, where only potent intervention might restore hormonal balance.[45]

These findings provide mechanistic insight into the involvement of the iNOS pathway in hypothyroidism-associated cardiorenal injury. While AG was used here as an experimental iNOS inhibitor, future translational efforts should focus on identifying clinically safe and effective strategies for modulating iNOS activity, rather than considering AG itself for therapeutic development.

Collectively, the results of this study demonstrate that AG exhibits substantial renal and cardio protective effects, likely mediated through its antioxidant, free radical scavenging and antifibrotic properties. For future studies, it would be useful to elucidate the intracellular signaling mechanisms implicated in anti-fibrotic and the upregulation of antioxidant enzymes by AG.

Conclusions

Using biochemical and histological experiments, it was shown that the iNOS inhibitor AG has anti-fibrotic and anti-oxidant properties in hypothyroidism-associated cardiovascular and renal injury in rats. Further research is needed to clarify the molecular mechanism (s) for the beneficial effect of AG renal and cardiovascular system function in hypothyroidism conditions.

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None

Author Contribution

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Competing Interests

No competing interests have been declared by the authors.

Ethical Approval

All experimental procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee of North Khorasan University of Medical Sciences (Ethics Code: IR.NKUMS.REC.1400.180).

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