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Full Length Research Article

Dysthyroidism Induces Hepatorenal Injury by Modulating HSP70/HSP90 and VEGF Signaling in Male Wistar Rats

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Summary: Thyroid hormones have been shown to promote the generation of reactive oxygen species (ROS), consumption of antioxidants, and induction of oxidative stress, which triggers the release of heat shock proteins (HSPs) and VEGF-dependent angiogenesis. The present study investigated the effect of altered thyroid states, hypothyroidism and hyperthyroidism, on hepatic and renal functions, oxidative stress biomarkers, and hepatorenal expressions of HSP70, HSP90, and VEGF. Male Wistar rats were randomized into vehicle-treated control, carbimazole-induced hypothyroidism, or levothyroxine-induced hyperthyroidism. Altered thyroid states caused impaired hepatic and renal functions accompanied by elevated malondialdehyde and reduced glutathione content and superoxide dismutase and catalase activities in the hepatic and renal tissues. These derangements were associated with down-regulation of hepatic and renal HSP70 and HSP90 and upregulation of hepatic and renal VEGF expression. Findings of histopathological examinations of the hepatic and renal tissues align with the biochemical derangements observed. This study reveals that dysthyroidism impairs hepatorenal function via induction of oxidative stress and modulation of HSP70/HSP90/VEGF signaling.

Keywords*: Hypothyroidism; hyperthyroidism; HSP70; HSP90; VEGF; oxidative stress*

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INTRODUCTION

Thyroid hormones (THs) are endocrine regulators of cellular activities such as thermoregulation and metabolism (Mariani and Berns, 2012; Ajayi *et al*. 2018a; Ajayi *et al*. 2018b). THs regulate cellular basal metabolism and oxidative processes (Klein and Danzi, 2007) by accelerating basal cellular metabolism, hence increasing metabolic reactions (Ajayi *et al*. 2017a). Also, they influence the electron transport chain, thus increasing oxygen consumption (Weetman *et al*. 1992). This promotes the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Weetman *et al*. 1992), consumption of antioxidants (Mano *et al*. 1995; Ajayi *et al*. 2017b), and induction of oxidative stress (Ajayi *et al*. 2017b; Guerrero *et al*. 1999; Akhigbe and Ajayi, 2021). The intracellular level of triiodothyronine (T3) determines the biological activities of THs. T3, produced via outer-ring 5-deiodination of thyroxine (T4) (Pavelka, 2014), binds with nuclear receptors of THs with a higher affinity than T4 (Asmaa *et al*. 2016). The liver and kidney are the main sites of the conversion of T4 to T3 (Brown *et al*. 2013) and are also affected by altered thyroid states (Ajayi *et al*. 2018b; Ajayi and Akhigbe, 2012; Ajayi *et al*. 2019). Although dysthyroidism has been reported to be an endocrine disruptor causing derangement of hormonal milieu and alteration in reproductive organ cytoarchitecture (Ajayi *et al*. 2013), most of the damages caused by dysthyroidism have been linked with oxidative stress (Ajayi *et al*. 2018a; Ajayi *et al*. 2018b; Ajayi *et al*. 2017a; Ajayi *et al*. 2017b; Pavelka, 2014).

Oxidative stress occurs when ROS generation exceeds cellular antioxidant buffering capacity (Akhigbe *et al*. 2021). This results in lipid peroxidation, protein denaturation, and oxidative DNA damage of the affected cell (Akhigbe and Ajayi 2021; Akhigbe *et al*. 2021; Akhigbe *et al*. 2020; Saka *et al*. 2020; Ige and Akhigbe, 2013). Besides the antioxidant system, the cells protect themselves by up-regulating the expression of heat shock proteins, HSPs, in response to ROS (Ikwegbue *et al*. 2018). HSPs response is primarily regulated through heat shock factor-1 (HSF-1), although post-transcriptional mRNA stabilization contributes some measure of regulation (Kaarniranta *et al*.

1998). HSPs synthesis is initiated in response to triggers such as physical, metabolic, and oxidative stress (Kaarniranta *et al*. 1998; Oosten-Hawle *et al*. 2013; Oksala *et al*. 2007). Subsequently, HSPs confer cytoprotection via chaperoning activities, including polypeptide folding, assembling, and translocation of organelles across membranes, conducting repairs, and degradation of irreparable peptides (Shiber and Ravid, 2014; Mayer and Bukau, 2005), and prevention of ROS-induced DNA fragmentation (Jacquier-Sarlin *et al*.1994). Hence, the antioxidant system works in synergy with HSPs to either prevent or neutralize the cellular impacts of ROS (Trott *et al*. 2008; Wu *et al*. 2015).

Despite the deleterious effects of oxidative stress on tissues, many compelling studies have established the positive role of oxidative stress in angiogenesis. Angiogenesis, either physiological or pathological, is activated by a rise in tissue demand for oxygen and nutrients, leading to hypoxia/re-oxygenation cycle and promotion of ROS generation (Kim and Byzova, 2013). Studies have shown that hydrogen peroxide induces vascular endothelial growth factor (VEGF) in vascular smooth muscle cells and endothelial cells, thereby enhancing angiogenic responses (Reuf *et al*. 1997; Chua *et al*. 1998). In addition, studies have revealed that ROS-mediated angiogenesis is linked with VEGF expression (Reuf *et al*. 1997; Li *et al*. 2010; Lu *et al*. 1998). ROS influence VEGF-stimulated VEGF receptor (VEGFR) 2 dimerization and autophosphorylation, required for VEGFR2 activation and angiogenesis (Ushio-Fukai *et al*. 2002; Colavitti *et al*. 2002). Hence, ROS has been established to promote angiogenic responses via modulation of VEGF/VEGF2 signaling.

Although we have previously demonstrated that dysthyroidism, hypothyroidism and hyperthyroidism, results in disruption of hepatic and renal cytoarchitecture, which is accompanied by impairment of hepatic and renal function (Ajayi *et al*. 2018b; Ajayi and Akhigbe, 2012; Ajayi *et al*. 2019), the roles of HSPs and VEGF in altered thyroid state-induced hepatorenal injury are unknown. Thus, the present study explores the role of HSPs and VEGF in dysthyroidism-induced hepatorenal injury.

MATERIALS AND METHODS

Ethical approval: The study was approved by the Ministry of Health Research Ethics Committee, Oyo State, Nigeria (reference number: AD13/479/460).

Experimental Animals: Male Wistar rats of comparable weight (230±20g) were used in this study. Animals were obtained from the Institute of Advanced Medical Research and Training, University College Hospital, Ibadan Nigeria. The animals were kept in wired mesh cages and acclimatized for two weeks before the commencement of the experiment. Animals had unrestricted access to standard rat pellet and water. The study was approved by the Ministry of Health Research Ethics Committee, Oyo State, Nigeria (reference number: AD13/479/460) and carried out following the Guide for the Care and Use of Laboratory Animals of the National Academy of Science (NAS), published by the National Institute of Health.

Experimental design: The rats were randomly allotted to three groups (n = 6): Control, Carbimazole-induced hypothyroid state, and Levothyroxine-induced hyperthyroid state. The control animals were administered 1 ml of distilled water as a vehicle. In contrast, carbimazole-treated animals received 20 mg/kg BW of carbimazole and the Levothyroxine-treated animals received 50µg/kg BW of levothyroxine. All treatments were via gavage and once daily for 35 consecutive days as previously reported (Ajayi *et al*. 2018a; Ajayi *et al*. 2018b; Ajayi *et al*. 2017a; Ajayi *et al*. 2017b; Ajayi and Akhigbe, 2012; Ajayi *et al*. 2019; Ajayi *et al*. 2013).

Sample collection: The Wistar rats were humanely culled under anaesthesia by administering 40 mg/kg of 5% ketamine and 4 mg/kg of 2% xylazine intraperitoneally (Ajayi and Akhigbe 2020). Blood samples were obtained through cardiac puncture into lithium-heparinized sample bottles and centrifuged at 3000 g for 5 minutes to obtain the serum. The liver and kidneys of each rat were excised, separated from surrounding structures, blotted, and weighed immediately. A weighed section of each organ was homogenized in an appropriate volume of cold Phosphate Buffer Solution. The homogenates were centrifuged at 12000 g for 15 minutes. The supernatant was separated into sample tubes, frozen overnight to maximize the release of the enzymes in the tissue.

Determination of hepatic and renal functions: Activities of hepatic aspartate aminotransferase (AST) (Agappe, India), alanine aminotransferase (ALT) (Randox Laboratory Ltd., Antrim, UK), and alkaline phosphatase (ALP) (Teco Diagnostics, USA) were used as indices of hepatic function and determined spectrophotometrically as previously documented (Saka *et al*. 2011; Hamed *et al*. 2021). Serum concentrations of creatinine and urea were determined using colorimetric methods (Randox Laboratory, Antrim, UK) (Saka *et al*. 2011; Hamed *et al*. 2021).

Determination of hepatic and renal redox markers: Colorimetry was used to determine the hepatic and renal levels of malondialdehyde (MDA) (Ajayi and Akhigbe 2020, Adegunlola *et al*. 2012), reduced glutathione (GSH) (Ajayi and Akhigbe 2020), and activities of superoxide dismutase (SOD) (Ajayi and Akhigbe 2020; Hamed *et al*. 2021) and catalase (Ajayi and Akhigbe 2020; Saka *et al*. 2011).

Determination of hepatic and renal HSP 70, HSP 90, and VEGF: Hepatic and renal concentrations of HSP70, HSP90, and VEGF were measured using ELISA kits (Elabscience, Biotechnology Co., Ltd, USA) per the manufacturer's guidelines.

Histopathological examinations: The harvested hepatic and renal tissues were fixed in 10% formalin immediately. The specimens were dehydrated in alcohol, cleared of xylene and embedded in paraffin. Seral sections were cut, stained, and examined under light microscope. Photomicrographs were taken at 400 x magnification.

Statistical analysis

Statistical analyses were carried out using GraphPad Prism (Versions 5). One-way analysis of variance (ANOVA)

followed by Tukey's posthoc test was used to compare the mean values across and between the groups. Values are presented as mean ± SD. *P* values < 0.05 was considered statistically significant.

RESULTS

Effects of dysthyroidism on hepatic and renal functions: Carbimazole-induced hypothyroidism caused a significant decrease in the activities of hepatic AST, ALT and ALP compared to the control and levothyroxine-induced hyperthyroidism (Figure 1). However, the activities of hepatic AST, ALT, and ALP were comparable between the control and hyperthyroid rats (Figure 1). Although serum creatinine concentration was similar across the groups, the serum urea concentration increased significantly in the hypothyroid and hyperthyroid rats (Figure 2).

Effects of dysthyroidism on markers of oxidative stress: There was a significant rise in the hepatic and renal concentrations of MDA in dysthyroid rats compared to the control group. The rise in hepatorenal MDA was significantly more prominent in hyperthyroid rats than hypothyroid rats (Figure 3A and 3B). Also, carbimazole-induced hypothyroidism and levothyroxine-induced hyperthyroidism caused a significant reduction in the hepatic and renal levels of GSH (Figure 3C and 3D). The activity of hepatic and renal SOD was significantly reduced in hypothyroid and hyperthyroid groups compared with the control. However, the decline observed in SOD activity was more pronounced in the hyperthyroid rats (Figure 3E and 3F). Similarly, hepatic and renal catalase activity was significantly suppressed in carbimazole-induced hypothyroidism and levothyroxine-induced hyperthyroidism compared with the control. This alteration was also observed to be more prominent in the hyperthyroid animals than the hypothyroid animals (Figure 3G and 3H).

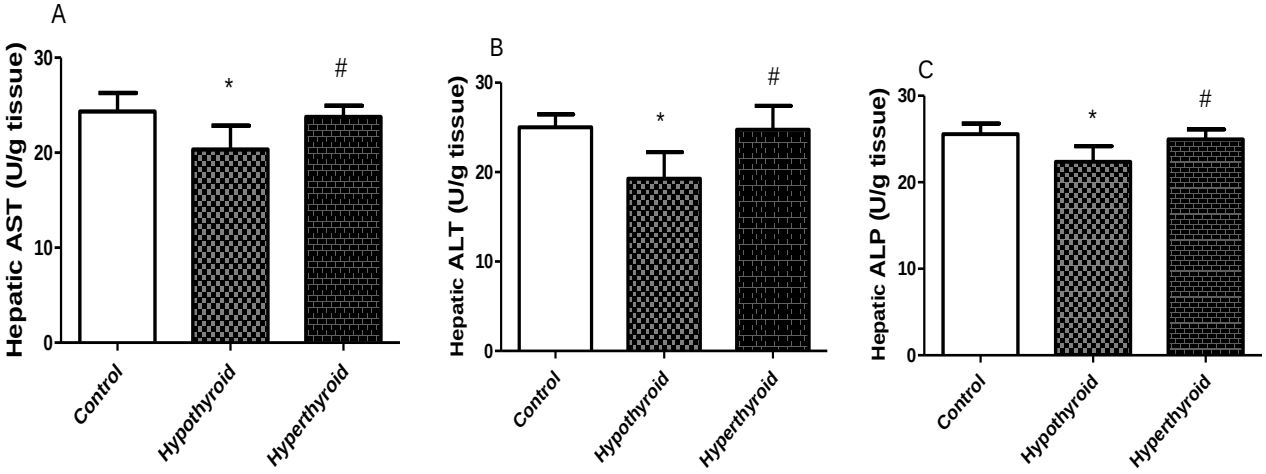

Figure 1:
Effect of hyperthyroidism and hypothyroidism on hepatic marker enzymes; aspartate amino transaminase (AST) (A), alanine transaminase (ALT) (B), and alanine phosphatase (ALP) (C). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. Values are expressed as mean ± SD of 5 replicates per group. *p<0.05 vs control, #p<0.05 vs hypothyroid.

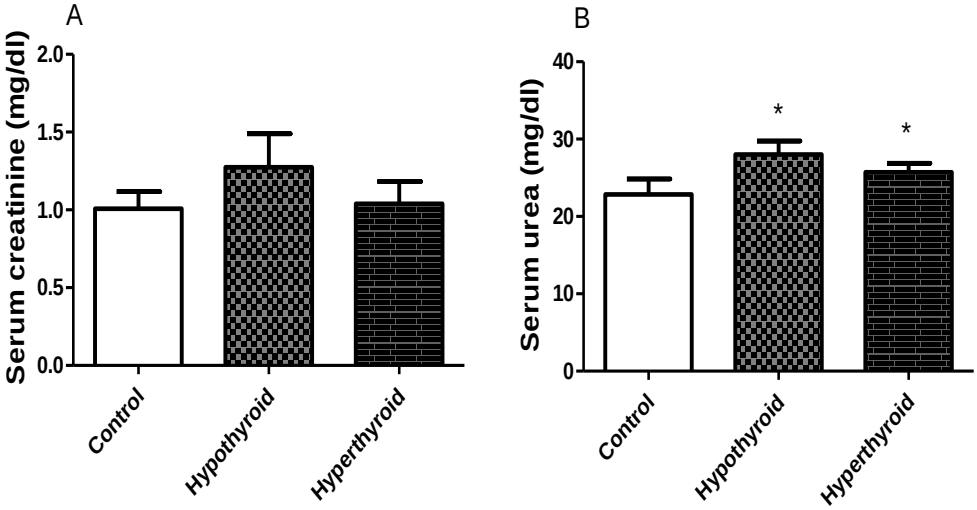

Figure 2:
Effect of hyperthyroidism and hypothyroidism on renal function markers; serum creatinine (A), and serum urea (B). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. Values are expressed as mean ± SD of 5 replicates per group. *p<0.05 vs control.

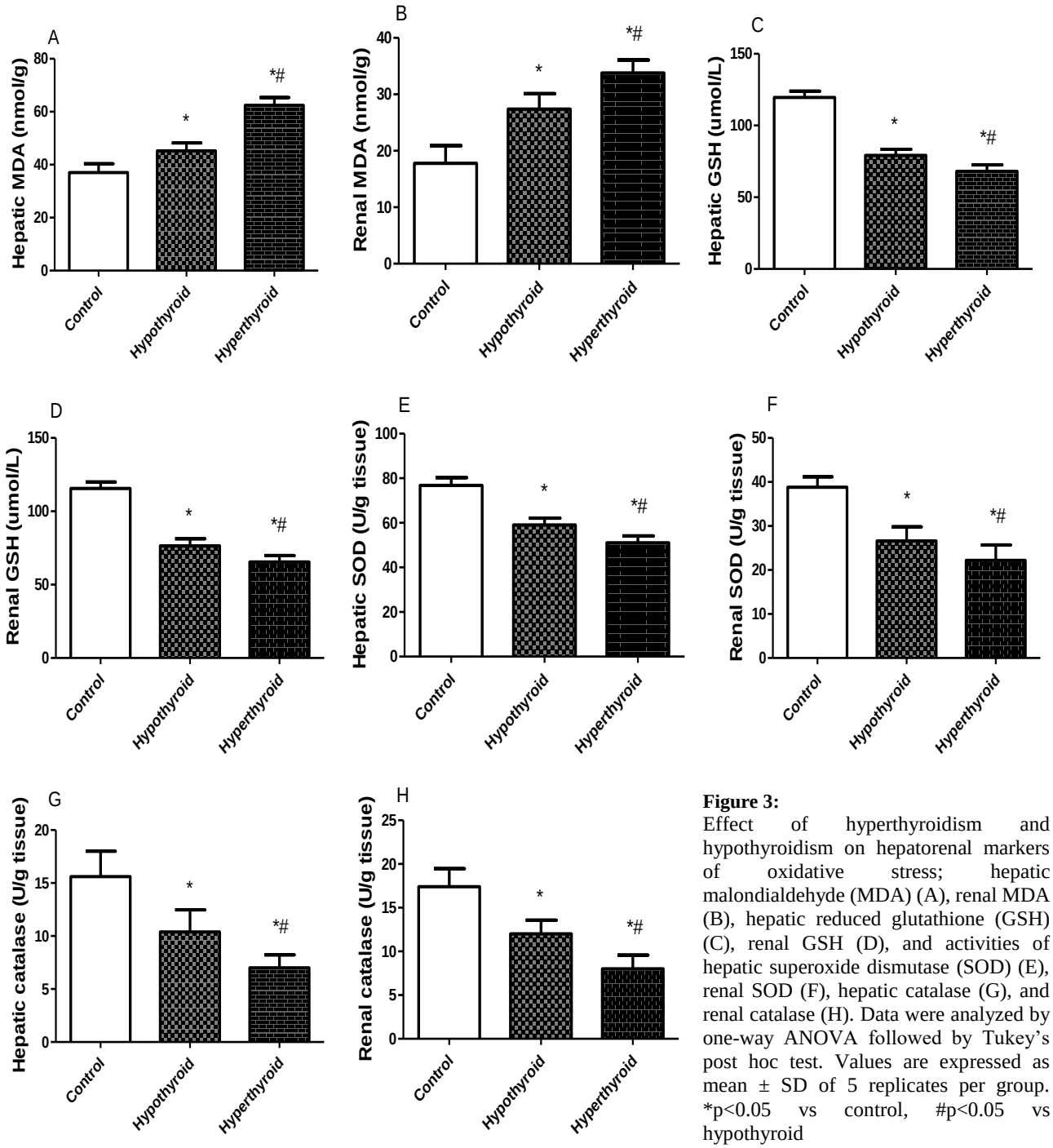

Figure 3:
Effect of hyperthyroidism and hypothyroidism on hepatorenal markers of oxidative stress; hepatic malondialdehyde (MDA) (A), renal MDA (B), hepatic reduced glutathione (GSH) (C), renal GSH (D), and activities of hepatic superoxide dismutase (SOD) (E), renal SOD (F), hepatic catalase (G), and renal catalase (H). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. Values are expressed as mean ± SD of 5 replicates per group. *p<0.05 vs control, #p<0.05 vs hypothyroid

Effects of dysthyroidism on HSPs: Hepatic HSP70 was comparable between the hypothyroid and hyperthyroid rats but significantly lower in hypothyroid and hyperthyroid rats when compared with the control (Figure 4A). In addition, carbimazole-induced hypothyroidism and levothyroxine-induced hyperthyroidism led to a significant reduction in renal HSP70. The observed reduction in renal HSP70 in dysthyroid states was significantly more prominent in the hyperthyroid rats (Figure 4B). There was a significant reduction in hepatic HSP90 in the dysthyroid rats when compared with the control; however, hepatic HSP90 was significantly higher in levothyroxine-induced hyperthyroidism than in carbimazole-induced hypothyroidism (Figure 4C). There was reduced expression of HSP90 in the renal tissues of dysthyroid animals when compared with the control. The observed reduction in renal HSP90 was similar between the hypothyroid and hyperthyroid rats (Figure 4D).

Effects of dysthyroidism on VEGF: Figure 5 depicts the effects of carbimazole-induced hypothyroidism and levothyroxine-induced hyperthyroidism on hepatic and renal VEGF. There was a significant rise in hepatic VEGF level in carbimazole-induced hypothyroidism and levothyroxine-induced hyperthyroidism compared with the control animals (Figure 5A). Compared with hypothyroid rats, hyperthyroid animals showed significantly higher hepatic VEGF levels (Figure 5A). Renal VEGF level was comparable in the hypothyroid and hyperthyroid animals, whereas renal VEGF level was significantly higher in hypothyroid and hyperthyroid animals when compared with the control group (Figure 5B).

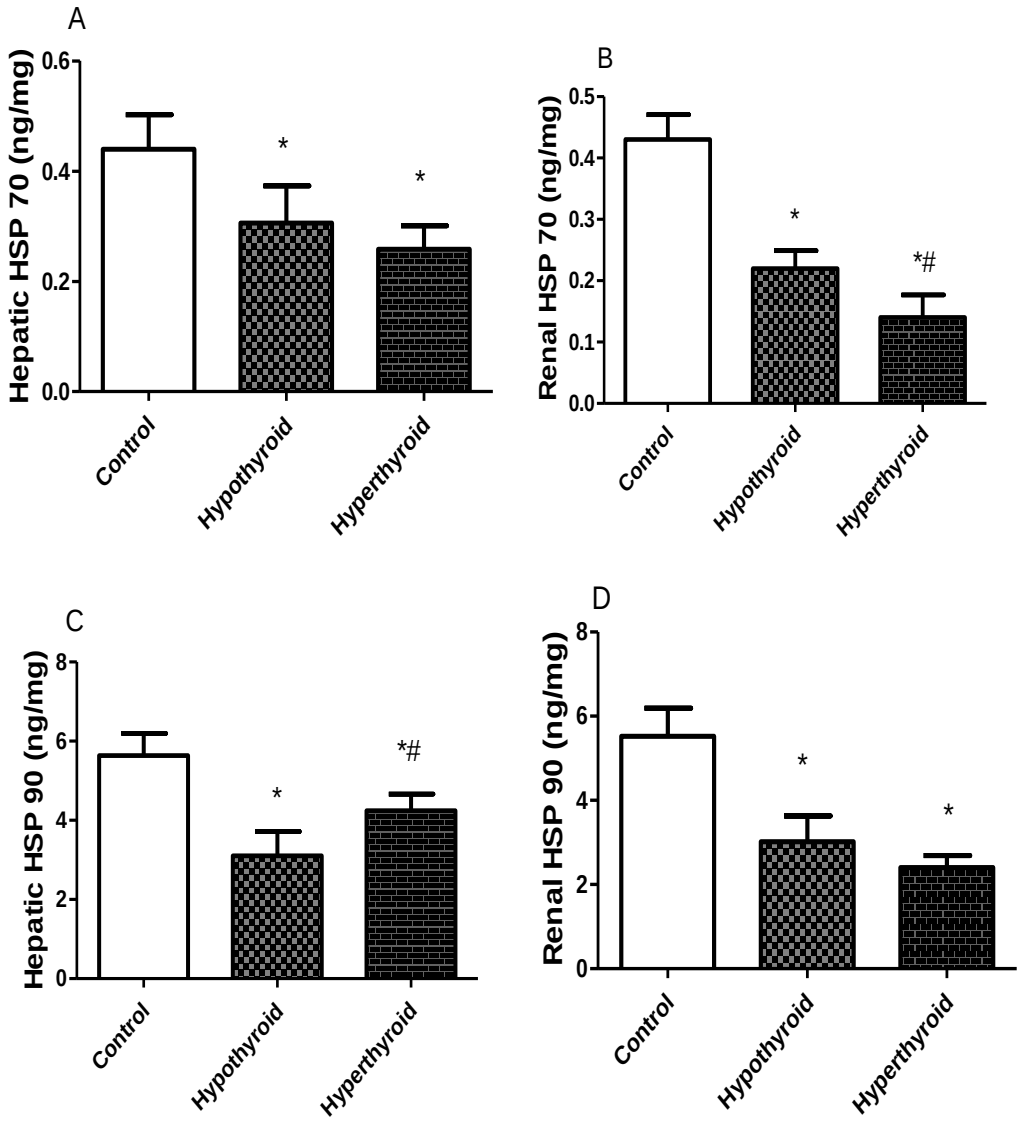

Figure 4:
Effect of hyperthyroidism and hypothyroidism on heat shock proteins (HSP); hepatic HSP 70 (A), renal HSP 70 (B), hepatic HSP 90 (C), and renal HSP 90. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. Values are expressed as mean ± SD of 5 replicates per group. *p<0.05 vs control, #p<0.05 vs hypothyroid.

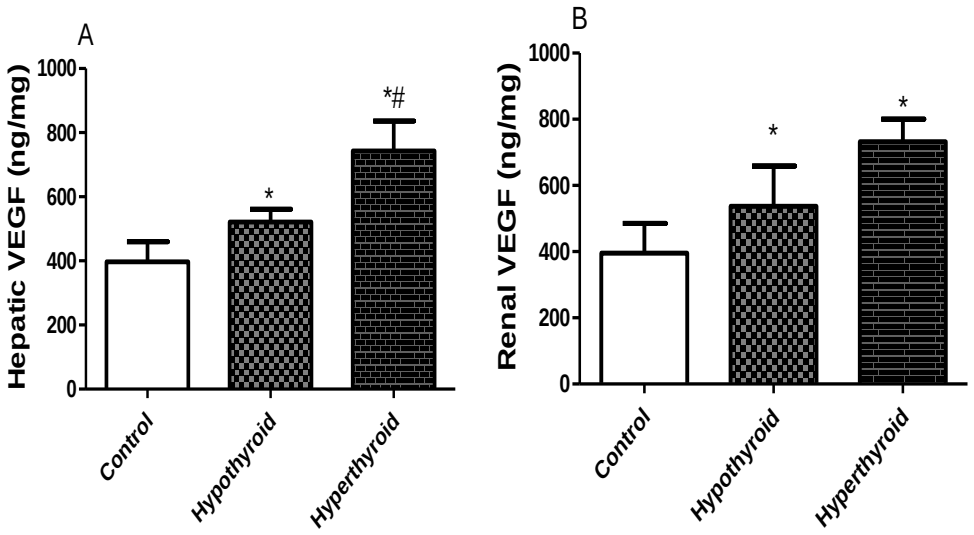

Figure 5:
Effect of hyperthyroidism and hypothyroidism on vascular endothelial growth factor (VEGF) in the liver (A) and kidney (B). Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. Values are expressed as mean ± SD of 5 replicates per group. *p<0.05 vs control, #p<0.05 vs hypothyroid.

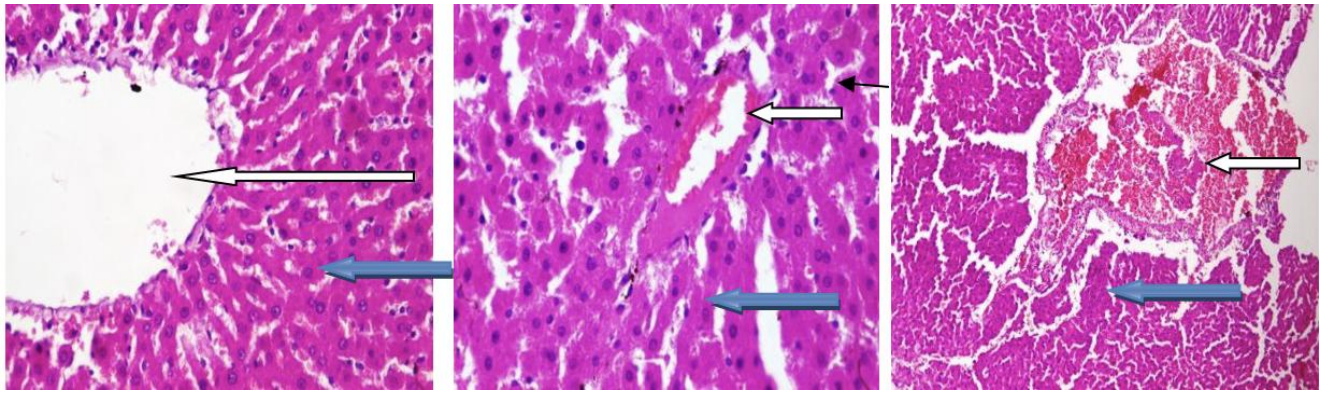

Plate 1

Photomicrograph of the liver section stained by Haematoxylin and Eosin. The control rats show normal central venules without congestion (white arrow). The morphology of the hepatocytes appear normal (blue arrow) and the sinusoids also appear normal without infiltration of inflammatory cell (slender arrow). The hypothyroid and hyperthyroid rats show central venules with mild congestion (white arrow). The morphology of the hepatocytes appear normal (blue arrow) and the sinusoids appear normal (slender arrow). Image is at 400 x magnification.

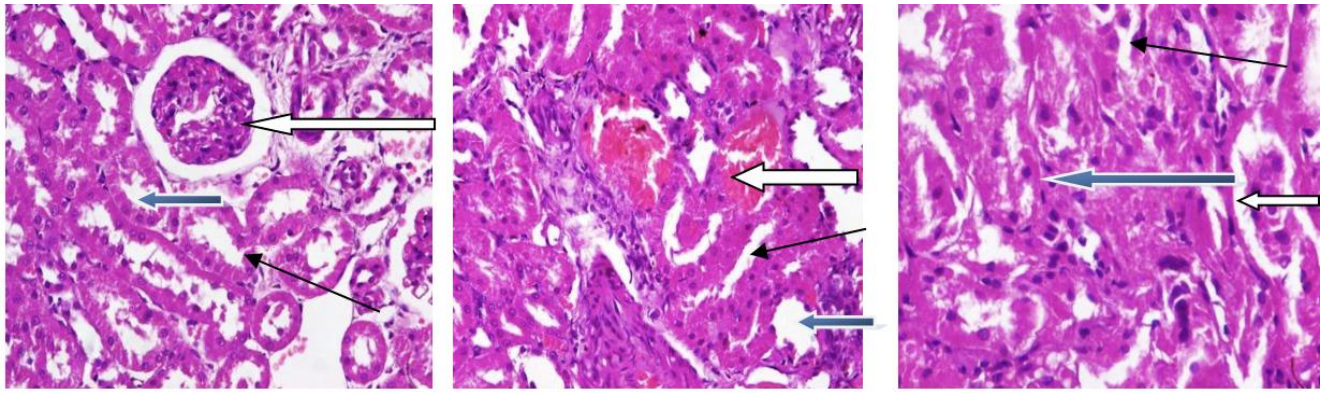

Plate 2:

Photomicrograph of the kidney section stained by Haematoxylin and Eosin. The kidneys of the control rats show normal architecture. The renal cortex show normal glomeruli with normal mesengial cells and capsular spaces (white arrow). The renal tubules including distal convoluted tubules and proximal convoluted tubules appear normal (blue arrow), and the interstitial spaces appear normal (slender arrow). The hypothyroid rats show moderate architecture. The renal cortex show normal glomeruli with normal mesengial cells and capsular spaces (white arrow). The renal tubules appear normal (blue arrow), and the interstitial spaces show mild vascular congestion with mild perivascular infiltration of inflammatory cells (slender arrow). The hyperthyroid rats show renal cortex with normal glomeruli with normal mesengial cells and capsular spaces (white arrow). The renal tubules show severe desquamation and severe tubular necrosis (blue arrow), and the interstitial spaces appear normal (slender arrow). Image is at 400 x magnification.

Effects of dysthyroidism on hepatorenal cytoarchitecture: The control rats showed presevred hepatic cytoarchitecture with normal central venules and no congestion (Plate 1). The morphology of the hepatocytes appeared normal and the sinusoids also appeared normal without infiltration of inflammatory cells. The hypothyroid and hyperthyroid rats showed central venules with mild congestion. The morphology of the hepatocytes appeared normal and the sinusoids appear normal. In addition, the renal tissue of the control rats showed normal architecture. The renal cortex showed normal glomeruli with normal mesengial cells and capsular spaces.

The renal tubules including distal convoluted tubules and proximal convoluted tubules appeared normal, and the interstitial spaces also appeared normal. The hypothyroid rats showed moderate architecture. The renal cortex showed normal glomeruli with normal mesengial cells and capsular spaces. The renal tubules appeared normal, and the interstitial spaces showed mild vascular congestion with mild perivascular infiltration of inflammatory cells. The hyperthyroid rats showed renal cortex with normal glomeruli and normal mesengial cells and capsular spaces. The renal tubules showed severe desquamation and severe tubular necrosis, and the interstitial spaces appeared normal (Plate 2).

DISCUSSION

In the current study, we explored the effects of altered thyroid states on hepatorenal function. We further determined the effects of dysthyroidism on hepatic and renal HSP and VEGF. It was hypothesized that the effects of dysthyroidism on hepatorenal function would be via HSP70/HSP90/VEGF-dependent pathway. Although we did not present the data on thyroid function test (thyroid stimulating hormone, TSH, T4 and T3) in the present report,

earlier studies in our laboratory have consistently established that 20 mg/kg of carbimazole and 50μg/kg of levothyroxine induce hypothyroidism and hyperthyroidism respectively in a rat model (Ajayi *et al*. 2018a; Ajayi *et al*. 2018b; Ajayi *et al*. 2017a; Ajayi *et al*. 2017b; Ajayi and Akhigbe, 2012; Ajayi *et al*. 2019; Ajayi *et al*. 2013). Our present data confirm that hypothyroidism and hyperthyroidism cause hepatorenal dysfunction. They also demonstrate that dysthyroidism induces oxidative stress in hepatorenal tissues evident by elevated hepatic and renal MDA and reduced GSH level and suppressed activities of SOD and catalase. These effects were accompanied by downregulation of hepatorenal HSP70 and HSP90 and upregulation of VEGF expression in the hepatic and renal tissues. These data suggest that hypothyroidism and hyperthyroidism induce hepatorenal dysfunction via oxidative stress and modulation of HSP70/HSP90/VEGF signalling.

As expected, the findings in the present study revealed that hypothyroidisim and hyperthyroidism caused hepatorenal dysfunction evident by significant alterations in marker enzymes of hepatic function and serum urea. This aligns with previous findings (Ajayi *et al*. 2018a; Ajayi *et al*. 2018b; Ajayi and Akhigbe 2012; Ajayi *et al*. 2019; Ellervik *et al*. 2019; Basu *et al*. 2012; Capasso *et al*.1999; Kim *et al*. 2020; Sequeira *et al*. 2011; Arora *et al*.2009), demonstrating hepatorenal dysfunction in dysthyroidism. In a human study, Ellervik *et al.* (2019) revealed that hypothyroidism reduced glomerular filtration rate (eGFRcrea) and increased incident chronic kidney injury. Similarly, Capasso *et al.* (1999) demonstrated that dysthyroidism led to perturbation of glomerular filtration rate and renal plasma flow through modification of proximal tubular sodium transport on Na/K-ATPase. Also, Arora *et al.* (2009) documented that hypothyroidism induces hepatorenal dysfunction by elevating hepatic marker enzyme activities and increasing serum creatinine and uric acid levels.

Although compelling shreds of evidence show that dysthyroidism induces hepatorenal injury, the associated mechanisms are yet to be fully explored. The finding that dysthyroidism is accompanied by oxidative hepatorenal damage is thus noteworthy. The lipid peroxidation induced by altered thyroid states is associated with a decline in the level of GSH in hepatorenal tissues and reduced activities of SOD and catalase in hepatic and renal tissues. This suggests the loss of the protective ability of SOD and catalase as enzymatic antioxidants in hepatorenal tissues in altered thyroid states. ROS act as signaling molecules at physiological concentrations and mediate a wide range of physiological processes, including homeostasis maintenance (Akhigbe and Ajayi, 2021). However, excessive ROS production is a key player in the initiation, progression and clinical outcomes of oxidative stress (Usman *et al*. 2019), resulting in pathological states such as organ dysfunction. The oxidation of the polyunsaturated fatty acid content of the membrane phospholipids (lipid peroxidation) in the cell membrane and membrane of cellular organelles triggers conformational changes that impair membrane and organelle functions (Akhigbe and Ajayi, 2021; Usman *et al*. 2019). This process leads to organ toxicity (Akhigbe and Ajayi, 2021; Awasthi *et al*. 2004) and cell death via the generation of MDA (Akhigbe and Ajayi, 2021). The observation of a rise in hepatic and renal MDA concentrations could infer that dysthyroidism triggers hepatorenal lipid peroxidation and possible cell death. The observed suppression of hepatic and renal activities of SOD and catalase provides further evidence that dysthyroidism enhances ROS generation (Weetman *et al.* 1992), antioxidants consumption (Mano *et al*. 1995; Ajayi *et al*. 2017b), and induction of oxidative stress (Ajayi *et al*. 2017b; Guerrero *et al*. 1999; Akhigbe and Ajayi, 2021).

HSPs are important sensors of cellular redox change and confer cellular protection in synergy with antioxidants. These sensors are triggered in response to oxidative stress (Kalmar and Greensmith, 2009) and act as molecular chaperones, promoting folding and inhibiting protein aggregation or targeting improperly folded proteins to specific degradative pathways (Oosten-Hawle *et al*. 2013; Kalmar *et al*. 2009). More so, HSP70 has been established to exert anti-inflammatory actions via prevention of the activation of NF-kB, cyclo-oxygenase 2 (COX-2), and nitric oxide synthase (NOS) (Kalmar and Greensmith, 2009). In addition, HSP70 and HSP90 inhibit apoptotic cascade by preventing the activation of caspase-3 by apoptosis protease activating factor-1 (Apaf-1) (Kalmar and Greensmith, 2009). Hence, the effects of HSPs go beyond the maintenance of protein folding-competent states. Kalmar and Greensmith (Kalmar and Greensmith, 2009) earlier suggested that most of the tissue damage that occurs due to oxidative stress occurs after the actual insult. Thus, it is plausible to infer that the observed low levels of HSP70 and HSP90 accompanied by a rise in MDA and reduced SOD and catalase activities in hepatic and renal tissues in dysthyroidism is a result of excessive accumulation of ROS and suppression of antioxidant with possible prevention of heat shock factor-1 (HSF-1), which is responsible for the biosynthesis and release of the cytoprotective HSPs. This possibly led to cell death and hepatorenal dysfunction observed. The present study found that dysthyroidism induces oxidative stress and suppression of HSPs in agreement with our previous study that demonstrated that dysthyroidism activates TNF-dependent inflammation and oxidative stress in rat cardiac tissue (Ajayi *et al*. 2017b), which could imply the loss of cytoprotective ability of HSPs in altered thyroid states. The observed distortion in the hepatorenal cytoarchitecture in dysthyroid animals could be explained by the loss of the protective effects of HSPs and resultant oxidative stress.

Earlier studies have reported that VEGF could be constitutively expressed or upregulated by oxidative stress (Klettner and Roider, 2009; Nagineni *et al*. 2003; Treins *et al*. 2001). VEGF is the primary physiological growth factor in angiogenesis. It has been reported to play a central role in the female reproductive cycle and wound healing (Klettner and Roider, 2009), maintenance of existing vasculature by inhibiting apoptosis of the endothelial cells (El-Remessy *et al*. 2004), and conferring neuroprotection in the eye (Zachary, 2004). Thus, the increase in hepatic and renal levels of VEGF observed in this study suggests that dysthyroidism causes pathological angiogenesis. A previous study has reported that inhibition of JNK promotes oxidative stress-dependent secretion of VEGF, while inhibition of p38 and Erk diminishes VEGF expression (Klettner and Roider, 2009). This could suggest that inhibition of JNK and activation of p38/Erk could cause

upregulation of VEGF expression. Taken together, therefore, our finding in this study that dysthyroidism leads to enhanced expression of VEGF in hepatic and renal tissues is noteworthy since it could infer that dysthyroidism could upregulate hepatorenal VEGF expression via modulation of mitogen-activated protein kinase (MARK) signaling (which includes JNK, p38, and Erk).

In conclusion, the current study demonstrates that hypothyroidism and hyperthyroidism cause hepatic and renal dysfunction and hepatorenal oxidative stress. These events are associated with suppression of HSP70 and HSP90 and upregulation of VEGF expressions in hepatic and renal tissues. These findings have far-reaching imports and could suggest alternative pathways for dysthyroidism-induced hepatorenal dysfunction. This study shows that modulation of HSP70/HSP90/VEGF signaling mediates dysthyroidism-induced oxidative hepatorenal damage.

Authors' contributions

Conceptualization and study design: AFA, REA, OSO
Experimentation: LOM, AG, OAI, ATI
Statistical analysis: AFA, REA
Writing of first draft: REA
Revision of the first draft: All authors
Approval for submission and publication: All authors

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