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# HYPOXIA ALTERS SIGNALING MOLECULES IN THE RENAL SYSTEM OF GREY MULLET SURVIVING IN POLLUTED ESTUARY- A COMPARATIVE STUDY

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**Abstract:** Physiological hypoxia, a condition associated with insufficient oxygen, is considered as the major consequence of xenobiotics stress and the renal system is highly sensitive to oxygen deprivation during its role in the elimination of toxic pollutants. Hence our concern is to monitor the key molecular markers such as hypoxia inducible factor 1 α (HIF1α), Endothelin 1 (ET1), Endothelial nitric oxide (eNOS), Hemeoxygenase - 1 (HO-1), and Heat shock protein 70 (HSP70) in fish (Mugil cephalus-grey mullet) kidney of unpolluted (Control-Kovalam estuary) and polluted (Test-Ennore estuary) site. Homogenate prepared from the test fish renal system depicted a significant increase in HIF1α, ET1, HO-1, and HSP70, along with a decrease in eNOS when compared to the control condition. It reveals that fish surviving polluted estuary is challenging hypoxic events which are well portrayed from the intense expression of HIF1α, ET1, HO-1 in the renal tissue homogenate. Thus, our study suggests that hypoxia response is one of the adaptive strategies elicited by Xenobiotic stress in the fish kidney, which may favor the upregulation of signaling proteins to mediate the fish survival in the polluted estuary.

***Key words:*** Fish, Kidney, Pollutants, Heat shock proteins, Hypoxia

## INTRODUCTION

Estuarine pollution with biologically active chemicals and xenobiotics due to anthropogenic activities from the burgeoning population has been a severe environmental problem all over the world in the last few decades. Ennore estuary is one such estuary, situated in the northern side of Chennai city frequently loaded with contaminants (Jayaprakash *et al*., 2005, Selvaraj *et al*., 2003) elicits the deterioration of both water quality and aquatic habitats of the Ennore estuary (Joseph *et al*., 1993).Our cohort studies witnessed that the water quality of Ennore estuary was highly deteriorated by the pollutants by the assessment of physico-chemical characteristics such as temperature, salinity, pH,biochemical oxygen demand (BOD), chemical oxygen demand (COD)and dissolved oxygen of Ennore estuarine water samples (Padmini and Usharani 2009). Thus, Ennore estuary represented as a polluted/test site.

In contrast, Kovalam estuary located in the Chennai south was chosen as the unpolluted/control site, due to free of polluting industries and comparatively less inflow of domestic sewage. Since, fish serves as an excellent bio-indicator, reflecting the impact of contamination in aquatic systems with higher sensitivity (Authman, 2015). Hence, Grey mullet was chosen as study organism.Previous studies from ourlaboratory recognized grey mullet as the reliable biomonitor, depicted from the differential biochemical response in liver, gills, brain, muscle and adipose tissue of fish from two different estuaries (Padmini *et al*., 2009a,b; Padmini and Usharani, 2011; Padmini and Vijaya Geetha, 2012; Padmini and Tharani, 2015; Padmini *et al*., 2016a,b).

The toxicants that enter the fish are mainly through

the gills, and consequently, with the blood, they reach the parenchymal organs where they retain for a longer time (Terra *et al*., 2008). Also, the toxicant concentrations are observed mainly in gills that reflect the pollutant concentrations in the water whereas, increased xenobiotics in water, the toxicant concentration is increased in other organs such as liver and kidney because they store toxicants (Kroglund *et al*., 2008). Heavy metals that escape from the liver after the completion of detoxification also enter the kidney, and therefore the Kidney is second to gill as an effector organ in ionic regulation and plays a vital role in the removal of the xenobiotics (Tamer *et al.*, 2018).

This has raised our concern to elucidate the biological response of the grey mullet's kidney as it functions as an essential excretory and regulatory organ. Fish kidney receives about 20% of the cardiac output and comparatively largest proportion of post-branchial blood, accompanied with the regulation of various physiological functions such as blood filtration, nitrogenous waste excretion, re-absorption of metabolites, regulation of acid-base levels, maintenance of osmotic balance, and the secretion of hormones (Reilly *et al*., 2007). Also, the kidney also plays a crucial role in the elimination of toxicants generated at the end of the physiological system's biological process (Paul et al., 2012). Hence our concern is to monitor the key molecular markers such as hypoxia inducible factor 1α (HIF1α), Endothelin 1, eNOS, Hemeoxygenase 1 (HO-1) and HSP70 in kidney homogenate of fish (Mugil cephalus-grey mullet) from unpolluted (Control-Kovalam estuary) and polluted (Test-Ennore estuary) site to understand the adaptive mechanism during hypoxia mediated by pollutants.

Elevated levels of xenobiotics create hypoxia in the aquatic environment (Rahman and Thomas, 2012). Hypoxia means low oxygen (oxygen concentration less than 2-3 mg/L), which can cause increased cellular oxidative stress with consequent damage to lipids, proteins, and DNA, resulting in severe cell damage(Tafani 2016). Kidney is highly vulnerable to hypoxia because the balance between the oxygen consumption and supply is critical as it requires high oxygen supply during its role in the elimination of toxic pollutants (Hansel 2013). Response to hypoxia is one of the mechanisms for kidneys to adapt to the oxygen-deficient condition and to survive under pathological conditions (Liu *et al*., 2017). Hypoxia-inducible factor-1 (HIF-1) is a critical molecular regulator of hypoxic stress and also an oxygen sensor in the fish kidney(Majmundar 2010). Hypoxia-inducible factors (HIFs) are key molecules that regulate various transcription factors involved in glucose transport, glycolysis, erythropoiesis, angiogenesis, vasodilation, and respiratory rate, and together they function to minimize the effects caused by low O2 at cellular, tissue and systemic levels (Ziello 2007). HIF-1 is a heterodimer protein complex composed of an alpha and a beta subunit(Berchner-Pfannschmidt, 2008). Under normoxia, multiple oxygen-dependent catalytic steps degrade HIF-1α subunits making the protein inactive. During hypoxia, however, degradation of HIF-1α is impaired, leading to accumulation and coupling of the HIF-1α -1α subunits, which allows for translocation of the protein to the nucleus and binding to hypoxia response element of DNA (Xiao *et al.*, 2013). When Hypoxia persists in the kidney, HIF signaling leads to adaptive responses to reduce oxygen demand and increase the oxygen supply needed for the maintenance of energetic homeostasis in tissues. Under hypoxia, Endothelin-1 induces HIF-1α by upregulation of HIF-1α synthesis and downregulation of Prolyl Hydroxylase Domain 2-mediated degradation (Francesca *et al*., 2010).

Endothelin is one of the most potent renal vasoconstrictors which play an essential role in the regulation of renal blood flow, glomerular filtration, sodium and water transport, and acid-base balance (Guan *et al*., 2015). ET-1, ET-2, and ET-3 are the three distinct endothelin isoforms comprising the endothelin family. The most crucial isopeptide is endothelin-1 (ET-1), which is produced by endothelial, mesangial, glomerular epithelial, and medullary collecting duct cells. Hypoxia induces the synthesis and secretion of Endothelin-1 from endothelial cells (Wang 2014). ET-1 is produced by almost every cell type in the kidney. ET-1 is the renal peptide that exerts its biological activity by binding to G-protein-coupled receptors ETA and ETB. It is commonly accepted that the endothelin stimulation of ETA receptors on smooth muscle cells leads to a

cellular contraction, whereas activation of ETB receptors in endothelial cells leads to NO production (Kowalczyk *et al.*, 2015).

It is interesting that ET-1 and Nitric Oxide (NO) work as negative feedback signals for each other, each one acting to limit the action of the other. NO, a potent vasodilatory substance is generated from endothelial nitric oxide synthase (eNOS). eNOS which converts L-arginine and molecular oxygen to L-citrulline and the vasodilator NO using O2 as well as electrons from NADPH and it is an essential regulator of vascular tone (Forstermann 2012).

Nitric oxide (NO) and NO donors were among the first reported inducers of the tissue-protective protein heme oxygenase-1 (HO-1) with a potential for eventual use in humans (Otterbein 2016). Heme oxygenase (HO) is the first rate-limiting enzyme that catalyzes the degradation of heme to biliverdin, iron, and carbon monoxide (CO) ( Ryter *et al*., 2009). Heme oxygenase-1 (HO-1) is the inducing form of HO and has been demonstrated to exert marked anti-oxidative and anti-inflammatory properties (Abraham 2008). Considerable evidence has demonstrated that HO-1 plays an essential role in the stress response to various pathological stimuli to serve a cytoprotective function.

Hypoxia plays a key role in inducing the HSPs such as HSP70and HO-1 in promoting the cytoprotective function and also demonstrating their differential expression during hypoxia (Chebotareva 2017).HSP70 protein induction is an exclusive biomarker of stress and/or, adaptive/cytoprotective function is likely a part of a broader, coordinated response to a hypoxic insult. HSP70, via its chaperonic role, facilitates the activation or repression of signaling proteins, which in turn regulates cell survival and apoptosis (Caroline 2000). The chaperonic function persuaded by HSP70 involves the initiation of protein folding, repair, refolding of misfolded peptides, and possible degradation of damaged proteins. A recent study by Wang *et al*., (2010) reported that HSP70 is a potential antiapoptotic factor and the induction of HSP70 is crucial for regulating renal cell survival and for preserving renal functions under hypoxia mediated renal injury.

## MATERIALS AND METHODS

### Study Site

Two estuaries were chosen as the experimental sites for the present study. Kovalam estuary (12°472 16" N, 80°142 58" E) is situated on the east coast of India, about 35 km South of Chennai. It runs parallel to the sea coast and extends to a distance of 20 km. It was chosen as the unpolluted site as it is surrounded by high vegetation, free from industrial or urban pollution. Ennore estuary (13°142 51"N, 80°192 31" E) also situated on the east coast of India, is about 15 km North of Chennai. It runs parallel to the sea coast and extends over a distance of 36 km, was chosen as the polluted site as in its immediate coastal neighbourhood are situated, a number of industries which include petrochemicals, fertilizers, pesticides, oil refineries, rubber factory and thermal power stations that discharge their effluents directly into this estuary (Padmini, E. and Vijaya Geetha, B. 2007a,b) (Vijayavel, KC. Anbuselvam, MP. 2006). Water quality was assessed by analyzing dissolved oxygen level of both Kovalam and Ennore estuary. Dissolved oxygen level was estimated by CHEMLINE portable dissolved oxygen meter CL-930 and it is expressed as ppm. Simultaneously fish were collected from both estuaries and placed immediately into insulated containers filled with aerated estuarine water at ambient temperature (25-30°C) and salinity (24-29 ppt).

### Study Animal and Sampling

M. cephalus a natural inhabitant of the estuaries, identified by the use of Food and Agriculture Organization (FAO) species identification sheets (Fischer, W. and Bianchi, G. 1984) was chosen as the experimental animal for the study. Grey mullets with an average length of 30 cm were collected from both Kovalam (n=20) and Ennore (n=20) estuaries using baited minnow traps. Collected fish were placed immediately in insulated containers filled with aerated estuarine water at ambient temperature and salinity. Fish were maintained in the above specified conditions for 4–5 hrs until the start of the experimental procedure for the isolation of kidney.

### Kidney Homogenate Preparation

The fish were stunned by a blow on the head, and a midventral incision was made to expose the kidney.

Fish kidneys were carefully scraped from the body cavity and washed with ice-cold saline blotted, weighed. One gram of kidney tissue was homogenized with 10 mL homogenizing buffer (Phosphate- buffered saline)(100 mM, pH 7.4) (usually 10 ml of PBS to 1g tissue) and the suspension was centrifuged at 4°C for 10 min at 10,000xg. The supernatant was used for the biochemical estimations.

**Quantification of HIF1α, ET1, eNOS, HO-1 and HSP70 using ELISA**

HIF1α, ET1, eNOS, HO-1 and HSP70 in fish kidney were quantified using respective ELISA kits (CSB-E12112H, CUSA BIOTECh, 96 T; MBS261099, Mybiosource USA; SEA868Hu ProLabMarketing Pvt Ltd NewDelhi; ADI-EKS-800, Biogeniux; MBS706016, MybiosourceUSA; according to the manufacturer's instruction.

**HIF1α**

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for HIF1α was already pre-coated onto a microplate. Standards and samples were pipetted into the wells, and HIF1α present were bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for HIF1α was added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) was added to the wells. A substrate solution was added to the wells after washing and color developed in proportion to the amount of HIF1α bound in the initial step. The color development was stopped, and the intensity of the color was measured in a microplate reader at 450 nm. HIF1α concentrations from the kidney homogenate were quantitated by interpolating absorbance readings from a standard curve generated with the calibrated HIF1α protein standard provided.

**Endothelin 1**

The level of ET1 in the kidney homogenate was quantified using ET1 ELISA kit (MBS261099, Mybiosource, USA) according to the manufacturer's instructions. The protein was diluted using a buffer and plated on the 96 well plates along with the serially diluted ET1 standard and incubated at 37°C for 120 mins. Add 100µL of Biotinylated detection Ab to each well and incubated for 37°C for 1hr. The contents were washed three times with diluted wash buffer. After aspiration, 100µL of HRP-Avidin conjugate was added and incubated at 37° C for 30mins. Following incubation, the wells were washed for 5 times with wash buffer and the contents aspirated. The 90µL of substrate solution was added to each well and incubated at 37°C for 15mins. The color developmentwas stopped with stop solution, which converted the endpoint color to yellow, and the color intensity was measured in a microplate reader at 450nm. The concentration of ET-1 present in the sample was calculated by plotting ET-1 standard curve. The concentration of ET-1 present in the sample was calculated from the standard calibration and values were expressed in terms of picogram/mg protein.

**NOS3 (eNOS)**

The level of eNOS in the kidney homogenate was quantified using eNOS ELISA kit (SEA868Hu, Prolab marketing Pvt Ltd, New Delhi) according to the manufacturer's instructions. The protein was diluted using a buffer and plated on the 96 well plates along with the serially diluted eNOS standard and incubated for 1hr at 37°C. Add 100µL of detection reagent A to each well and incubated for 1hr at 37°C. The contents were washed three times with diluted wash buffer. After aspiration, 100µL of detection reagent B was added and incubated for 30 mins at 37°C. Following incubation, the wells were washed for 5 times with wash buffer and the contents aspirated. The 90µL of substrate solution was added to each well and incubated at 37°C for 10-20mins. The color development was stopped with stop solution, which converted the endpoint color to yellow, and the color intensity was measured in a microplate reader at 450nm. The concentration of eNOS present in the sample was calculated by plotting eNOS standard curve. The concentration of eNOS present in the sample was calculated from the standard calibration and values were expressed in terms of picogram/mg protein.

**HSP70**

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit was already pre-coated with an antibody specific to HSP70. Standards or samples were added to the appropriate microtiter plate wells with biotin-conjugated HSP70. A competitive inhibition reaction was launched between HSP70

(Standards or samples) and biotin-conjugated HSP70 with the pre-coated antibody specific for HSP70. The more amount of HSP70 in samples, the less antibody bound by biotin-conjugated HSP70. After washing, avidin conjugated Horseradish Peroxidase (HRP) was added to the wells. The substrate solution was added to the wells. Then the color development was stopped, and the intensity of the color was measured in a microplate reader at 450 nm. HSP70 concentrations from the sample were quantitated by interpolating absorbance readings from a standard curve generated with the calibrated HSP70 protein standard provided.

**HO-1**

The HO-1, ELISA kit is a quantitative sandwich immunoassay. The antibody specific for HO-1 was precoated on the wells of the provided HO-1 immunoassay Plate. HO-1 was captured by the immobilized antibody and was detected with HO-1 specific, polyclonal antibody. The polyclonal antibody was subsequently bound by a horseradish peroxidase-conjugated anti-IgG secondary antibody. The assay was developed with tetramethylbenzidine substrate, and a blue color develops in proportion to the amount of captured HO-1. The color development was stopped with acid stop solution which converts the endpoint color to yellow. The intensity of the color was measured in a microplate reader at 450 nm. HO-1 concentrations from the sample were quantitated by interpolating absorbance readings from a standard curve generated with the calibrated HO-1 protein standard provided.

Data were analyzed using statistical software package version 7.0. Student's t-test was used to ascertain the significance of variations between unpolluted and polluted fish kidney homogenate. All data were presented as mean ± SD of 20 samples. Differences were considered significant at $p<0.01$ and $p<0.001$.

## RESULTS AND DISCUSSION

The water quality parameters such as temperature, salinity, pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD) and dissolved oxygen of Ennore estuary have been assessed already and the results established that hypoxia persists in Ennore estuary due to the accumulations of contaminants, is the pioneer work from our laboratory. Aquatic hypoxia is frequently associated with changes in temperature, food availability, or pollutant exposure, each of which may interact with oxygen-dependent responses in fish. Availability of oxygen is the most important environmental factors that influence fish biodiversity, particularly in the coastal waters and estuaries. Variations in oxygen availability lead to biochemical adaptations in aquatic animals to overcome the consequences of oxidative stress. Fish may display metabolic depression as part of an adaptive response to a stress situation (e.g., hypoxia), resulting in decreased metabolic activity by multiple signaling factors at the tissue and cellular levels. Hypoxia inducible factor (HIF-1) is a transcription factor that acts as a global regulator of oxygen homeostasis and is part of the adaptive response to hypoxia (Semenza, 2009). Jessica and her colleagues (2014) have already reported that HIF1α levels were found to be increased during hypoxia in the gills of Ruffe and the Flounder. In fish (Percafluviatilis), elevated levels of HIF-1α were detected in the brain, eye, heart, and spleen during hypoxia (Rimoldi *et al.*, 2012). The gills are known for the prime site of gas exchange in fish, and therefore it is the first target in the event of oxygen depletion. Other than respiration, the gills are involved in ion exchange and acid-base regulation. Likewise, next to gills, the kidney is a major site of systemic oxygen sensing, and also in maintaining the body homeostasis by regulating the concentration of many of the plasma constituents, and by eliminating all the metabolic wastes. Hence, it is indirectly affected by pollution through blood circulation, and it appears to be particularly sensitive to a variety of toxic elements.Increased expression of HIF-1α (Fig-1) was observed in kidney homogenate inhabiting in Ennore estuary not only depicts the hypoxic condition of the estuary and also the adaptive mechanism exert by HIF-1α by regulating the transcription factors involved in cellular homeostasis, energy metabolism, glucose metabolism, angiogenesis, erythropoiesis, iron metabolism, pH regulation, apoptosis, growth, cell proliferation and biological functions. Cells in the kidney utilize various molecular pathways that allow them to respond and adapt to changes in renal oxygenation.

Renal hypoxia that principally reflects enhanced oxygen consumption for tubular transport leads to

intensified endothelin-1 (ET-1) generation through hypoxia inducible factor-(HIF) mediated transcription of pre-pro-ET-1 and endothelin-converting enzyme-1 (ECE-1) (Samuel 2018). ET-1 is intimately involved in the renal function, modulating glomerular filtration rate (GFR), solute and water reabsorption along the nephron, and renal acid excretion(Richter 2006).ET-1 is produced in the killifish gill, and that ET1 levels are regulated by environmental salinitywhich may affect the multiple functions of the gill, including regulating blood flow or ion transport (Hyndman et al., 2007). Concomitantly, a significant increase in the expression of ET1 (Fig-2) was ascertained in the kidney homogenate of grey mullet from Ennore estuary demonstrate that it may affectsvariety of biological functions in the kidney, such as constricting renal vasculature, inhibiting sodium and water reabsorption, and leading to glomerular and tubular damage.

Similarly, endothelial cells have several roles in the kidney, but the Endothelial nitric-oxide synthase (eNOS) –NO pathway is the most predominant pathway in endothelial function(Sogawa 2018). eNOS is important in the control of blood vessel tone and remodeling, hemostasis, angiogenesis, and the mobilization of endothelial progenitor cells. Also, eNOS is implicated in numerous aspects of renal vascular control and function. eNOS, based on its generation of nitric oxide, is generally considered protective against renal injury. Earlier reports have revealed that the impaired availability of NO, caused by either genetic defects or inhibition of eNOS, promotes the progression of renal dysfunction. Hypoxia elicits endothelial dysfunction, in part, through reduced expression of eNOS (Heiss, 2015). Although a bunch of data has been generated supporting that deficiency of eNOS and bioavailability of NO accelerated the vascular, glomerular, and tubular injury. Renal homogenates of *P. dolloi* (Fresh water fish), revealed that reduction of eNOS-dependent NO production at the glomerular level decreases both the filtration pressure and the glomerular filtration surface, thus decreasing GFR (Amelio 2018). Similarly, in the present study, the reduced expression of eNOS (Fig-3) in the kidneys from Ennore estuary depicts the endothelial dysfunction and also dissembles the regulation of proximal Na+ and water reabsorption.

Also, hypoxia modulates several cellular metabolic functions, including the stress response. One of the stress response proteins is heme oxygenase-1 (HO-1) (Choi and Alam, 1996), a rate-limiting enzyme in heme catabolism. HO-1 is an inducible gene whose transcription is increased in response to a variety of cellular stresses and stimuli, including ischemia, hypoxia, oxidative stress, and inflammatory cytokines (Ferrandiz and Devesa, 2008). And HO-1 also participates in the maintenance of renal blood flow, vasotonic balancing, and sodium and fluid absorption in the loop of Henle. HO-1 induction is an indicator of oxidative stress, and HO-1 protein overexpression protects the kidney from free radical-mediated injury (Ozbek, 2012). Up-regulation of HO-1 reduces blood pressure due to an increase in the level of CO and bilirubin, a potent antioxidant. Robust HO-1 overexpressioninhibited inflammatory responses, presumably by a decrease in cellular heme-mediated oxidative stress and vasoconstrictors (Abraham 2009). Consistent with these observations, this study demonstrate that increased expression of HO-1 (Fig-4) in fish kidney homogenate from Ennore estuary might play a significant protective role in cells due to its ability to restore the redox health of the tissue in response to hypoxic stress.

Many different extrinsic and intrinsic apoptotic stimuli, including hypoxia, induce the accumulation of Heat shock proteins (HSPs) in the cells. The HSPs have a protective function that enables the cells to survive, excluding lethal conditions (Lanneau *et al.*, 2008). A regulatory link exists between the oxygen-sensing and the heat-shock pathways. This link involves the hypoxia-dependent up-regulation of the heat-shock factor because of the direct binding by HIF-1$\alpha$ that is necessary for full HSP induction during hypoxia (Baird *et al.*, 2006). Sanders and Martin (1993), have already reported that the levels of HSP60 and HSP70 are elevated in the mussels and fish tissue collected from the polluted areas. To the best of our knowledge, this study is the first study ascertained the expression of HSP70 in the fish kidney of field condition. Results depict that the level of HSP70 was increased (Fig-5) in the kidney homogenate of the polluted (Ennore) estuary involved

in cytoprotectionby maintaining the renal homeostasis. This is also supported by Yoo and Janz (2003) that the induction of HSP70 is a crucial biomarker of environmental stress, such as xenobiotics exposure, and also suggested that this HSP70 overexpression is involved in imparting cytoprotective mechanism via protein chaperoning to regulate the expression of apoptotic/antiapoptotic signaling proteins.

To sum up, our findings show hypoxia response is one of the adaptive strategies elicited by environmental stress in the fish kidney, which may favor the upregulation of signaling proteins to mediate the fish survival in the polluted estuary.

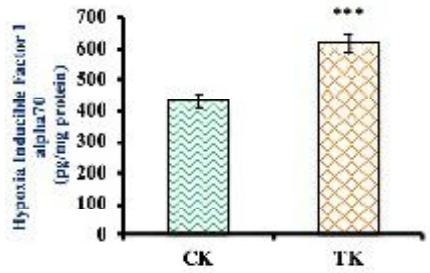


**Fig. 1.** Expression of HIF1α

HIF1α is expressed in the Figure 1. It depicts the increased expression of HIF1α (43%; p<0.001) in test kidney homogenate than in control kidney homogenate.

CK- Control Kidney homogenate, TK- Test Kidney homogenate

NS-not significant; $^{*}$ $p < 0.05$; $^{**}$ $p < 0.01$; $^{***}$ $p < 0.001$

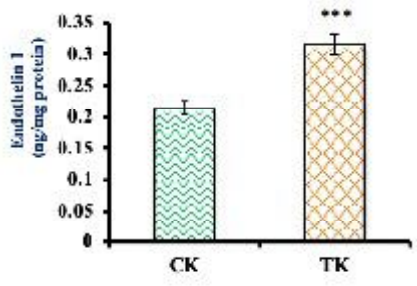


**Fig. 2.** Expression of ET1

Expression of **ET1** is presented in Fig. 2. **ET1** expression increased significantly (48%, $p < 0.001$) in test kidney homogenate than in control kidney homogenate.

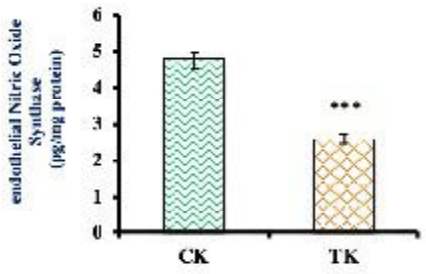


**Fig. 3.** Expression of eNOS

Expression of **eNOS** is presented in Fig. 3. **eNOS** expression decreased significantly (46%, $p$ <0.001)in test kidney homogenate than in control kidney homogenate.

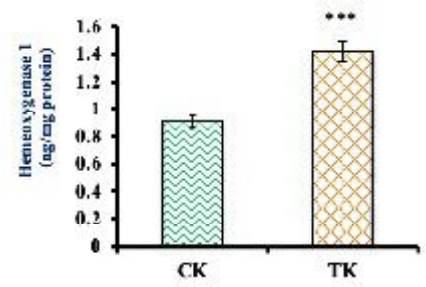


**Fig. 4.** Expression of HO-1

Expression of HO-1 is presented in Fig. 4. HO-1 expression increased significantly (56%, $p < 0.001$) in test kidney homogenate than in control kidney homogenate.

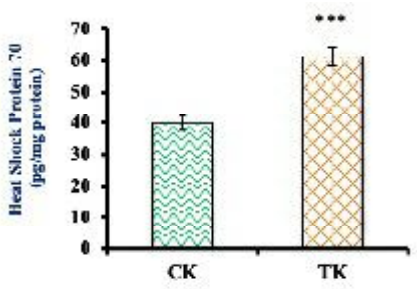


**Fig. 5.** Expression of HSP70

Expression of HSP70 is presented in Fig. 5. It depicts an increase in the expression of HSP70 (53%, $p < 0.001$) in test kidney homogenate than in control kidney homogenate.

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