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Effect of zinc on growth hormone receptor gene expression in liver of albino rats

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Abstract
The present investigation was carried out on 20 day-old albino female rats for 42 days to see the effect of zinc supplementation and zinc deficiency on expression of growth hormone receptor gene in liver tissue of albino rats. The animals were divided into three groups, *viz.* zinc control (ZC), zinc-supplemented (ZS) and zinc-deficient (ZD). Each group consisted of 20 animals. The ZC group received basal diet (BD) which contained zinc @ 7.80 ppm. The ZS rats were given BD (7.80 ppm), supplemented with zinc @ 32.20 ppm (total 40 ppm zinc), whereas the ZD rats received BD (7.80 ppm zinc) plus 1.3 % of calcium carbonate (to induce zinc deficiency) in the diet on dry matter basis. The animals were fed *ad libitum* throughout the investigation. Liver tissue collection was done on two occasions, i.e., on the 21st and 42nd day of the investigation. The relative expression of GHR gene in liver of rats was highest (2.55 ± 0.18 folds) in ZS rats (P<0.01) followed by ZC (1.01 ± 0.03 folds) and the lowest (0.35 ± 0.05 folds) in ZD rats in the first liver tissue collection (21st day of the investigation). In the second collection (42nd day of the investigation), the same trend of GHR gene expression was observed in which it was significantly (P<0.01) higher in ZS group (2.25 ± 0.14 folds) as compared to that of ZC (1.02 ± 0.08) and ZD (0.40 ± 0.6) rats. There was no significant difference in GHR gene expression in liver tissue between two collections (21st and 42nd day of the investigation) within group in all the treatment groups.

Keywords: growth hormone, gene expression, liver, albino rats

Introduction
Zinc plays an indispensable role in life. It is a vital micronutrient essential for the cellular events in microorganism (Sugarman, 1983) [12], plants (Broadley *et al.* 2007) [2] and animals (Prasad, 2008) [11]. The basic mechanisms of action of zinc are intimately linked to the structure and action of countless enzymes; but biochemical mechanisms involved are not totally known (Neto *et al.* 1995) [8]. Zinc is involved in the normal functions of more than 300 enzymes and approximately 2,800 proteins are potential Zn-binding proteins (Andreini *et al.* 2006) [1], consistent with an important role for Zn in biological functions. Therefore, zinc is fundamental for cell growth, development and differentiation. Zinc plays a dynamic role as an extracellular and intracellular signalling factor, which enables communication between cells in an autocrine, paracrine, or endocrine fashion, conversion of extracellular stimuli to intracellular responses, and regulation of various intracellular signalling pathways. These zinc functions are recognized as "zinc signalling," which has critical roles in physiology, and thus their imbalance can cause a variety of problems with regard to health (Kambe, 2014) [4].

Materials and Methods
A total of 60 numbers of female albino rats, 20 days old with uniform weight were purchased from Ghosh Enterprise (Govt. approved supplier), Kolkata, West Bengal. The animals were divided equally into three groups, *viz.* zinc control (ZC), zinc-supplemented (ZS) and zinc-deficient (ZD). The ZC group received basal diet (BD) which contained zinc @ 7.80 ppm. The ZS rats were given BD (7.80 ppm), supplemented with zinc @ 32.20 ppm (total 40 ppm zinc), whereas the ZD rats received BD (7.80 ppm zinc) plus 1.3 % of calcium carbonate (to induce zinc deficiency) in the diet on dry matter basis. All animals were fed *ad libitum* throughout the investigation period (42 days). Liver tissue was collected after slaughtering of 6 animals each from all groups on two occasions i.e., on 21st and 42nd day of the investigation under RNAse - free environment.

The collected tissue samples were immersed immediately in RNA ladder separately in RNAs-free appendorf tube labelled and kept at - 80ºC for quantitative polymerase chain reaction (qPCR). RNA extraction kit was purchased from Fermentas Company and the total RNA extraction was performed from the collected liver tissue under RNAse free environment as per the protocol given in the kit. For cDNA preparation, the Revert Aid cDNA Synthesis Kit was purchased from Thermo Scientific Company and the protocol given in the kit was followed.

Table 1: Primer sequences for GHR gene target analyzed in liver of rats by quantitative real-time PCR

Gene	Primer	Sequence (5'→ 3')
GHR	Forward	TCTCCAGCGACATGTTTCCT
	Reverse	AAAGGTCTCCAGTTCAGGGG
Beta Actin	Forward	GGCCCCTCTGAACCCTAAG
	Reverse	GGTACGACCAGAGGCATACA

The relative expression of GH1 gene in liver tissue was quantified and compared by Step One Plus™ model Real-Time PCR System (Manufacturer: Applied Bio system) using Step one software. Beta actin was used as a housekeeping gene. Each sample was tested in triplicate in 96 multiwell plates. The oligonucleotides for qPCR were designed on the basis of prior sequence information from other animals and basic local alignment search tool (BLAST) analysis against the rat genome (Table 1). The following components (total volume 10 µL) were added to the reaction mixture as follows:

cDNA	:	1 µL
Primer (forward)	:	0.5 µL
Primer (reverse)	:	0.5 µL
SYBR green master mix (2x)	:	5 µL
Nuclease free water	:	3µL

Similarly, beta actin reaction mixture (total volume 10 µL) was added as follows:

cDNA	:	1 µL
Primer (forward)	:	0.5 µL
Primer (reverse)	:	0.5 µL
SYBR green master mix	:	5 µL
Nuclease free water	:	3µL

A no template control (NTC) reaction (total volume 10 µL) was included for GHR and Beta actin gene and added as follows.

Primer (forward)	:	0.5 µL
Primer (reverse)	:	0.5 µL
SYBR green master mix (2x)	:	5 µL
Nuclease free water	:	4 µL

The relative expression of all the target genes was calculated using $2^{-\Delta\Delta C}{}_T$ formula as per Livak & Schmittgen (2001) [5].

Results and Discussion

The relative expression of GHR gene in liver of rats was the highest (2.55 ± 0.18) in ZS rats (P<0.01), followed by ZC (1.01 ± 0.03) and the lowest (0.35 ± 0.05) in ZD rats in the first liver tissue collection (21st day of the investigation). In the second collection (42nd day of the investigation), the same trend of GHR gene expression was observed where it was significantly (P<0.01) higher in ZS group (2.25 ± 0.14 folds) as compared to that of ZC (1.02 ± 0.08) and ZD (0.40 ± 0.6) rats. There was no significant difference in GHR gene expression in liver between collections (21st and 42nd day of the investigation) within group in all the treatment groups (Table 2; Fig. 1).

Table 2: Relative gene expression (folds) of GHR gene in liver (mean ± se) of zinc-supplemented and zinc-deficient rats

Treatment Tissue collection	ZC	ZS	ZD	p value
1st collection	1.01 ± 0.03 ^{b}	2.55 ± 0.18 ^{a}	0.35 ± 0.05 ^{c}	0.002**
2nd collection	1.02 ± 0.08 ^{b}	2.25 ± 0.14 ^{a}	0.40 ± 0.6 ^{b}	0.000**
p value	0.940 ^{NS}	0.963 ^{NS}	0.215 ^{NS}	

Means bearing different superscripts (a, b, c) in a row differ significantly (P<0.01).

The impaired growth induced by zinc deficiency in rats is associated with reduced plasma IGF-I (Ninh *et al.* 1995; McNall, 1995) [7, 9], hepatic GHR (Ninh *et al.* 1995; McNall, 1995) [7, 9] and serum GH binding protein (GHBP) levels, together with a decrease of their hepatic mRNA (Ninh *et al.* 1995) [9], that ultimately reduce somatic cell proliferation (MacDonald, 2000) [6]. GH infusion in zinc-deficient rats normalized liver GHR but failed to restore normal plasma IGF-I and growth (Ninh *et al.* 1998) [10]. The growth retardation caused by zinc deficiency is associated with defects in the GHR signalling pathway **(**McNall, 1995) [7] because zinc deficiency induced a state of GH insensivity (Yu *et al.* 2005) [13]**.** Another hypothesis is that zinc depletion might accelerate the degradation of GHR or alternatively induce translational stalling of the mRNAs **(**Ninh *et al.* 1995) [9].

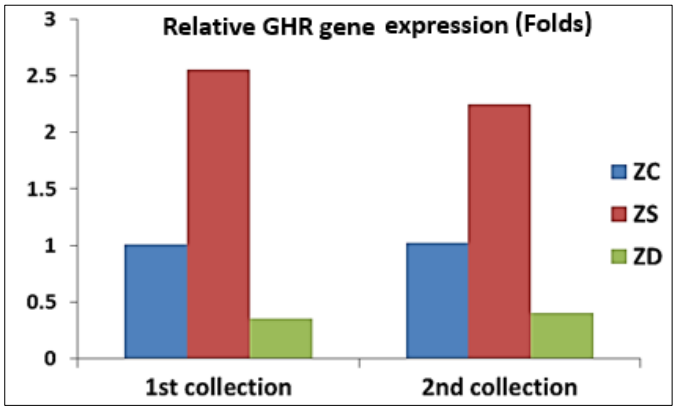

Fig 1: Relative gene expression (folds) of GHR gene in liver of zinc-supplemented and zinc-deficient rats

The mechanisms that account for the decrease expression of the hepatic GHR genes in zinc deficiency are obscure. One can postulate the existence of direct mechanisms such as those occurring for the MT gene by which extracellular zinc availability is directly responsible for the gene regulation (Dunn *et al.* 1987) [3]. Zinc deficiency markedly affects the expression of genes involved in the GH intracellular signalling pathway (McNall *et al.* 1995) [7] that might affect GHR gene expression. However, further investigation is needed to study the effect of zinc deficiency on gene transcription rates and resolve whether zinc affects the stability of GHR transcripts. The transcription rate of the GHR and /or mechanisms that regulate alternative splicing of GHR mRNA transcript may be affected by zinc deficiency.

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