Indian J Med Res 139, June 2014, pp 927-932

Histopathological changes due to the effect of selenium in experimental cockerels

S.A.A. Latheef[*,†], K. Radhika[**] & G. Subramanyam[††]

Departments of [*]Cardiology & [**]Pathology, Sri Venkateswara Institute of Medical Sciences, Tirupati, India

Received November 7, 2012

***Background & objectives*: Selenium usually acts as an antioxidant at optimal levels in the body and increased levels are toxic. In this study an attempt was made to evaluate the effect of an optimum dose (0.14 mg) of selenium on histopathological changes in experimental hypercholesterolemia in cockerels.**

***Methods*: The effect of selenium (0.14 mg) was investigated on histopathological changes in four tissues namely liver, kidney, heart, and descending aorta in cockerel animal model. Animals were either fed with stock diet (group C), stock diet with cholesterol (group CH), stock diet with selenium (group Se), stock diet, selenium and cholesterol (group CH+Se) for six months. Animals were sacrified and the tissues were isolated and subjected to histopathological study.**

***Results*: Xanthochromatic collections in liver were observed in group CH; hydropic degeneration in group Se and lobular disarray, hydropic degeneration and kuppfer cell hyperplasia in group CH+Se were observed. In kidney, mild mononuclear infiltration was observed in interstitium in groups CH, Se and CH+Se. myocyte disruption, and mononuclear infiltration in group CH and CH+Se, and disruption of muscle bundles with vascular congestion in group Se were observed. Smooth muscle proliferation in the media of blood vessel was observed in groups CH, Se and CH+Se.**

***Interpretation & conclusions*: The results of the present study suggested that the optimum dose of (140 µg/day) feeding induced atherogenesis by inflammation and smooth muscle proliferation in cockerels with experimentally induced hypercholesterolaemia.**

Key words Atherogenesis - cholesterol - cockerels - selenium

Trace elements are essential for normal human development and functioning of the body[1]. In biological systems, these trace elements are mostly bound to proteins, forming metalloproteins. Some of the metals in these metalloproteins are part of enzymatic systems, and have structural and storage functions[2]. Abnormalities in the metabolism of several trace elements have been reported in the pathogenesis of human diseases[2,3]. With the lifestyle modifications, many countries are facing increasing rates of cardiovascular diseases (CVD) including heart disease, hypertension, hyperlipidaemia, *etc*[4].Low levels of selenium can contribute to heart failure, and being deficient in selenium seems to make atherosclerosis worse[5]. But studies have shown that

Present addresses: [†]Post Doctoral Fellow, Department of Biochemistry, University of Hyderabad, Hyderabad 500 046, India
[††]Director, Narayana Medical College & Hospital, Nellore, India

selenium supplements do neither seem to have any effect on the progression of heart disease, nor do they protect against heart attack[6]. Selenium, supplementation (1 ppm) has been shown to increase LDL receptor activity and m-RNA expression[7]. Selenium concentrations were found to be inversely associated with the coronary heart disease and dilated cardiomyopathy in observational studies and the evidence from a few randomized studies is still inconclusive[8,9]. We have shown that 1mg of selenium supplementation elevated serum lipid profile in experimental rabbits[9,10]. Evaluation of these changes at the tissue level may reveal the mechanistic role of metal. Therefore, an attempt was made to study the effect of 0.14mg of selenium supplementation on histopathological changes in various organs in experimental cockerels.

Material & Methods

This study was conducted in the department of Pathology, Sri Venkateswar Institute of Medical Sciences (SVIMS), Tirupati, India during June 2000 to December 2000. The present study was undertaken on 40 male white Leghorn chicks (*Gallus domesticus)* weighing 674±93.37g. These chicks were procured from commercial poultry farm. The animals were maintained in the laboratory and were divided into four groups:

Group C (control): 10 cockerels were fed with stock diet of 100g per day (diet source: Krishna Poultry Farm, Tirupati, Andra Pradesh, India).

Group CH: 10 cockerels were fed with stock diet of 100 g + 100 mg of cholesterol + 1g butter per day; (cholesterol source: SRL, Mumbai, India and butter source: Amul India).

Group Se: Ten cockerels were fed with stock diet of 100g + 140 µg selenium as selenite per day (sodium selenite soruce: SRL, Mumbai) and

Group CH+Se: Ten cockerels were fed with stock diet of 100 g + 100 mg cholesterol + 1g butter + 140µg selenium as selenite per day.

The study protocol was approved by the Institutional Ethical committee, SVIMS, Tirupati.

These cockerels were fed for six months and no adverse clinical effects were observed during the period. After six months, the animals were sacrificed and organs (liver, kidney, heart, and descending aorta) were isolated. Tissue sections were made from each organ fixed on 10 per cent neutral buffered formalin. The sections were washed and dehydrated with graded ethanol series in the processor. After processing, tissue sections were embedded in paraffin blocks. Sealed sections were made using an automated microtome with a thickness of 4-5µm. Sections were stained with hematoxylin and eosin (H& E), and photographed under light microscope (Nikon, 463170, Japan) with attached camera (Nikon, 1103473, Japan).

Results

No abnormalities were detected in the liver, kidney and blood vessel but mononuclear infiltration in the heart of control group was observed. In liver, xanthochromatic collections in group CH; hydropic degeneration in group Se and lobular disarray, hydropic degeneration and kuppfer cell hyperplasia in group CH+Se were observed (Fig. 1a-d). In kidney, mild mononuclear infiltration in interstitium in groups CH, Se and CH+Se was observed (Fig. 2a-d). Myocyte disruption, and mononuclear infiltration in heart tissue in group CH and CH+Se and disruption of muscle bundles with vascular congestion in group Se were observed (Fig. 3a-d). Smooth muscle proliferation in the media of blood vessel was observed in group CH, Se and CH+Se (Fig. 4a-d).

Discussion

Selenium enters the environment from both geochemical and anthropogenic sources. Much of selenium in the environment comes from selenium dioxide produced by burning of coal and other fossil fuels. Inhalation of selenite and selenium dioxide can produce injury to respiratory tract, the cardiovascular and peripheral vascular system, brain, muscle, kidney and liver[11]. Acute Se toxicity is indicated by breath with garlic odour, dyspnoea, vomiting, and respiratory failure. Pathological changes in various organs include, congestion in the liver with areas of focal necrosis, congestion in the kidney, endocarditis, myocarditis petechial haemorrhages of the epicardium[12]. Chronic toxicity is indicated by growth inhibition and mortality, liver damage, splenomegaly and enlarged pancreas[13]. Food is the main source of Se for man, the dietary intake mainly depends on the region of origin of the food stuffs and the protein content[14].

In this study, the effect of selenium was investigated on histopathological changes in four organs namely liver, kidney, heart and blood vessel in control against cholesterol, selenium and selenium with cholesterol fed cockerels. In selenium exposed liver, hydropic

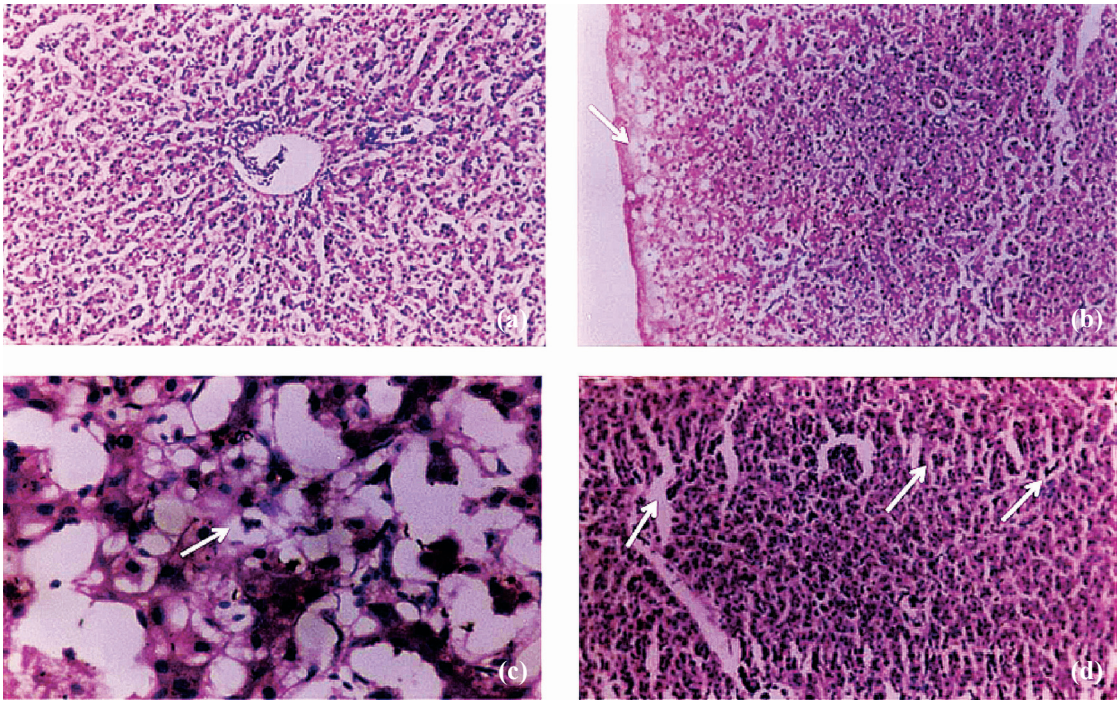

Fig. 1(a). Group C - Liver (Control) (H&E 4×10). **(b).** Group CH - Liver (H&E 4 × 10) - Xanthochromic collections (arrow). **(c)**. Group Se - Liver (H&E 10 × 10) - Hydropic degeneration. **(d)**. Group CH+Se - Liver (H&E 4 × 10) Lobular disarray, (I^{st} from the left arrow) hydropic degeneration (II^{nd} from left arrow) & kupfer cell hyperplasia (III^{nd} from left arrow).

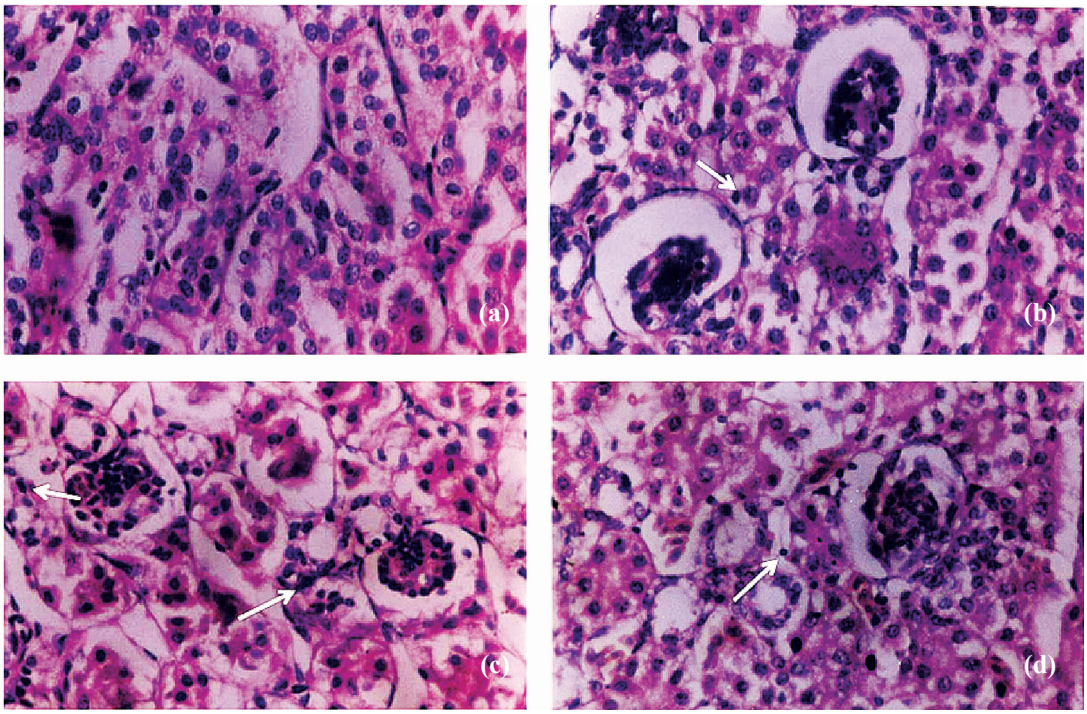

Fig. 2(a). Group C - Kidney (Control) (H&E 10 × 10). **(b)**. Group CH - Kidney (H&E 10 × 10) - Mild mononuclear infiltrate in interstitium. **(c)**. Group Se - Kidney (H&E 10×10) - Mild interstitial inflammation (arrows). **(d)**. Group CH+Se - Kidney (H&E 10×10) - Mild mononuclear infiltrate interstitium.

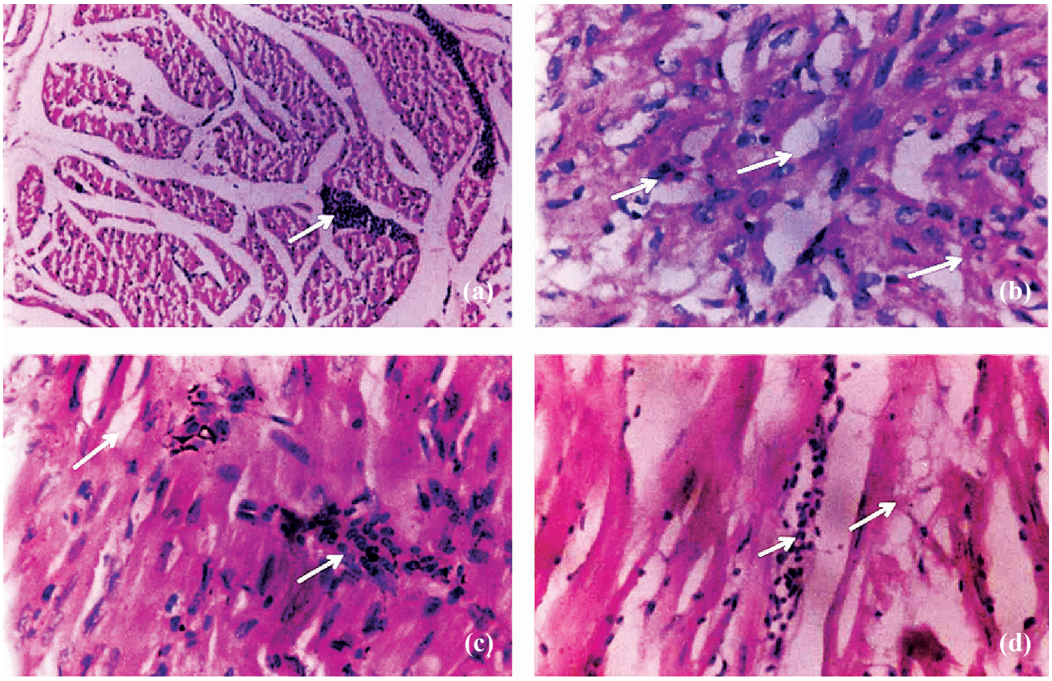

Fig. 3(a). Group C - Heart (Control) (H&E 4 × 10) - Mild mononuclear infiltrate. **(b)**. Group CH - Heart (H&E 10 × 10) - Myocyte disruption (1st from left arrow), cholesterol clefts, (IInd from left arrow), and mononuclear infiltration. (IIIrd from left arrow), **(c)**. Group Se - Kidney (H&E 10×10) - Disruption of muscle fibres (upper arrow) and mononuclear infiltration (lower arrow). **(d)**. Group CH+Se - Heart (H&E 10×10) - mononuclear infiltration (1st from left arrow) and Myocyte disruption (IInd from left arrow).

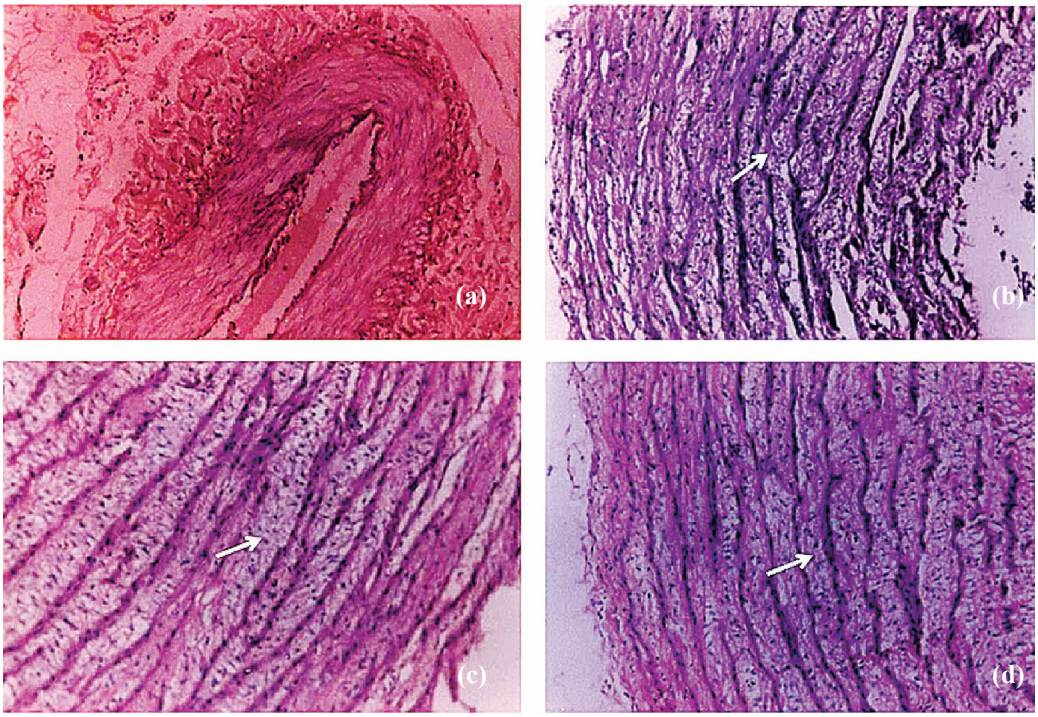

Fig. 4(a). Group C - Descending aorta (Control) (H&E 4 × 10). **(b)**. Group CH - Descending aorta (H&E 40 × 10) - Smooth muscle cell proliferation in the media. **(c)**. Group Se - Descending Aorta (H&E 40×10) - Smooth muscle cell proliferation in the media. **(d)**. Group CH+Se - Heart descending aorta (H&E 40×10) - Smooth muscle cell proliferation in the media.

degeneration was observed which suggested liver injury induced by the selenium. Hydropic dengeration occurs as a result of ion and fluid homeostatis leading to an increase in intraceullar water[15]. Mononuclear infiltration in interestititum of kidney in selenium exposed group was observed in the present study. Infiltration of mononuclear cells was observed in innermost cortex of pig kidney in hypercholesterolaemic condition where microvascular density was more[16]. It is suggested that these cell by secreting cytokines and growth factors induce new vessel growth thereby interfering with the regulation and/or spatial distribution of intrarenal blood flow, resulting in renal disease progression[16].

Monocytes disruption in heart tissue as observed in groups Se and CH+Se animals suggested the inflammatory changes in which monocyte chemoattracnt protein-1 (MCP-1) has been implicated to play an important role in many conditions including atherosclerosis[17]. Under conditions of chronic systemic inflammation, mediators derived from the myocardium may also participate in the pathogenesis of heart disease[18]. Smooth muscle proliferation in the media of blood vessel in selenium exposed group of animals was observed in the present study. Smooth muscle cells are found in media of the blood vessel with low proliferative index and in the process of atherogenesis, their proliferation is increased in the innermost part of media and intima[19]. Movement of medial cells into intima is a single, although initiating, step in the series of events which relate arterial smooth muscle to the atherosclerotic process[20].

The results of present study show that selenium induces atherogenesis via inflammation. The mechanisms of toxicity of selenium reported in the literature are redox cycling of auto-oxidisable selenium metabolites, glutathione depletion[21], protein synthesis inhibition, depletion of S-adenosyl-methionine (cofactor for selenide methylation), general replacement of sulphur and reactions with critical sulphydryl groups of proteins and cofactors[19]. Observational and randomized clinical trials have also raised concern that high selenium exposure may lead to adverse cardiometabolic effects, at least in selenium replete populations[22]. The optimum dose of Se is 140 µg, potential toxic dose is 800 µg per day in cockerels[23]. The safe dietary intake in humans is approximately 800 µg per day and the lethal dose is 5 mg[24]. The present results showed that the optimum dose of Se (140 µg/day) induced atherogenesis with cockerels with experimentally induced hypercholesterolaemia.

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Reprint requests: Dr Radhika Kottu, Associate Professor, Department of Pathology
Sri Venkateswara Institute of Medical Sciences, Tirupati 517 507, India
e-mail: kotturadhika@yahoo.com