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Impaired Antioxidant Enzyme Activities in Kidney and Liver Tissues of Hypertension Induced and Vanadium Treated Rats

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Abstract
Hypertension is a complex disorder that affects various organs of the body. The vital target of the present research was to study the effect of hypertension induction and vanadium treatment On antioxidant status in various tissues of the rat. In the present work, four domains of rats were taken i.e., Control domain, fructose-induced hypertension domain, vanadium treated domain, hypertension-induced + vanadium treated domain. The actions of antioxidant enzymes SOD (Superoxide dismutase), Lipid peroxidation, catalase, Glutathione peroxidase (GPX) were estimated in the kidney and liver of all the four domain rats. The result reveals that hypertension induction significantly reduced the levels of SOD, lipid peroxidation and in Contrast the catalase, glutathione peroxidase levels in both kidney and liver were significantly increased whereas the treatment with sodium met vanadate effectively reestablished the alterations in the antioxidant enzymes in both kidney and liver tissues of the rat.

Keywords
Hypertension, kidney, liver, SOD, catalase, GPX, lipid peroxidation.

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1. INTRODUCTION
Hypertension is one of the global problems [1] and especially in India [2] which is directly or indirectly responsible for a large cardiovascular disease, including coronary infarction and stroke. Oxidative stress results in the alteration of antioxidative defense systems, which is one of the fundamental factors for hypertension [3]. There are many natural and physiological factors that lead to oxidative stress, which will enhance the risk of hypertension for example, exposure to environmental oxidants, toxins such as heavy metals, ionizing and ultraviolet irradiation, lack of exercise, heat shock, inflammation, High salt intake, obesity, stress, depression, calcification and especially insulin resistance etc. [4-7]
Heart, Kidney, brain, liver and arterial blood vessels are reported to be the primary organs affected by hypertension [8]. Oxidative stress in the liver and kidney is a complex mechanism, involving intraorgan generation of reactive oxygen species. It leads to significant changes in cell components such as DNA, proteins, and cell membrane lipids. To reduce these damaging effects, organs maintain an oxido /redox balance by enzymatic and non-enzymatic components to detoxify excess Reactive oxygen species (ROS) [9-14]. Bhanot *et al.*, [15] reported that vanadium compounds caused marked and sustained decrease in plasma insulin concentration and blood pressure in rats. Bis (maltolato) Oxo vanadium was reported to reduce sucrose induced elevation of systolic blood pressure as well as some of the genetic hypertension [16]. The main aim of the present study is to use Vanadium to treat the hypertension and to study its effect on antioxidant enzymes in kidney and liver tissues of the rat.

2. MATERIALS AND METHODS

This study was undertaken as per the guidelines of the institutional research and ethics committees. Chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and plastics from Nunclon (Roskilde, Denmark).

2.1 Experimental Design:

For this experimental study 24 Albino male rats, weighing 0.2kg ±0.05 were used. Blood pressure was measured and Blood Samples (pre-experimental) were collected before starting the experiment later the rats were divided into the following domains. Domain-I: 6 rats were nourished with a standard diet (rat diet) procured from the National Institute of Nutrition (NIN) Hyderabad, India and normal for drinking water. Domain-II: 6 rats nourished with standard diet and supplemented with 0.5mg/kg of elemental vanadium as sodium meta vanadate. Domain-III: 6 rats nourished with standard diet and 20% fructose solution as drinking water. Domain-IV: 6 rats nourished with standard diet and 20% fructose solution as drinking water and supplemented with 0.5mg/kg of elemental vanadium as sodium meta vanadate. These rats were fed for 45 days and Systolic and Diastolic Blood pressure were noted. After 45 days the rats were sacrificed, blood samples (post-experimental) were collected, kidney and liver tissues were isolated and used for further analysis.

2.2 Superoxide dismutase activity (SOD) (E.C. 1.15.1.1):

One unit of enzyme activity was measured because of the inhibition of image reduction of nitro blue tetrazolium (NBT) by the protein as per the method of Beachamp and Fridovich [17]. The urinary organ i.e., kidney and liver tissues were homogenized (10% w/v) in potassium phosphate buffer hydrogen ion concentration (P^H 7.5) contain 1% polyvinyl pyrolidine and centrifuged at 16000 rpm for quarter hour. The supernatants were used as protein resource. Each tube consisting of the full reaction mixture of the hundred millimeter phosphate buffer (P^H 7.5),

10 mM EDTA, 130 mM methionine, 750 mM NBT, 60 mM riboflavin and also the protein sample. The reaction was initiated by the addition of sixty millimeter B complex and also the samples were placed underneath visible radiation light for half-hour and the ensuing color was determined at 560nm against the chemical agent, blank kept in dark place. The activity of the protein was expressed as units/ mg protein/ min.

2.3 Catalase activity (E.C. 1.11.1.6):

Catalase activity was resolute by a rather changed version of Aebi (1984) [18]. To 100 µl of excretory organ and liver tissue extracts, Ethyl alcohol (10 µl; 100%)was accessorial so placed in an ice bathtub for half an hour. Then the tubes were unbroken at temperature followed by the addition of ten µl of Triton X-100 RS. In every cuvette containing two hundred µl of phosphate buffer and fifty µl of tissue extract, it 250 µl of 0.066 M H_2O_2 (in phosphate buffer) was accessorial and reduce in optical density was measured at 240 nm for sixty seconds. 43.6 M cm-1 of the molar extinction constant was used to adapt to protein activity. One unit of activity is capable of the moles of H_2O_2 degraded/mg protein/min

2.4 Glutathione peroxidase (GPx; EC. 1.11.1.9)

Glutathione peroxidase was assayed by the strategy of Rot ruck *et al*. [19] both tubes consists 0.2 mL buffer, 0.2 mL EDTA, 0.1 mL metallic element compound (sodium azide), to its homogenates of excretory organ and liver tissue (homogenized in 0.4M phosphate buffer, pH 7.00) of 0.2 mL were added. To every mixture, 0.2 mL glutathione, followed by 0.1mL oxide were added. The contents were mixed well and incubated at 37°C for ten minutes in conjunction with a bearing tube containing all reagents however no accelerator. Once ten minutes, the reaction was inactive by the addition of 0.4 mL 100 percent TCA. The tubes were centrifuged and therefore the supernatant was assayed for glutathione content by using Ellman's chemical agent. The activity of peroxidase was expressed as millimole of GSH utilized/min/mg protein

2.5 Lipid peroxidation

The level of lipid peroxidation within the liver and excretory organ tissues were measured in terms of malondialdehyde (MDA; a product of lipid peroxidation) content and determined by using the thiobarbituric acid (TBA) chemical agent. The reactivity of TBA is decided with minor modifications of the tactic adopted by Osafumi and Hiroshi [20]. The tissues (liver and kidney) were homogenized (10% W/V) in 15% potassium chloride solution. Every tube a pair of 2.5 milliliters of material, 0.5 milliliter of saline (0.9% metal chloride), and 1.0 milliliter of (20% w/v) trichloracetic acid (TCA) was additional. For twenty minutes the contents were centrifuged on a cold centrifuge at 4000 x g. To them 1.0 milliliter of supernatant, 0.25 milliliter of TBA chemical agent were additional and therefore the contents were incubated at 95°C for one hour. One milliliter of n-butanol was additional to them. When thorough combining, the contents were centrifuged for quarter-hour at 4000 x g in a very cold centrifuge. The organic layer was transferred into transparent tubes and its ensuring color was measured at 532 nm. The acceleration of lipid peroxidation was

indicated as µ moles of malondialdehyde formed/g wet wt. of tissue.

3. RESULTS

3.1 SOD activity in Kidney

The SOD activity was significantly lower in a hypertension induced domain (Fig. 1a). There was no significant difference between the control domain, vanadium treated domain and hypertension+vanadium treated domain (Fig. 1a).

3.2 SOD activity in Liver

The SOD activity was significantly higher in the vanadium treated domain and significantly lower in the hypertension induced domain than any other domain (Fig. 1b). There was no significant difference between control and hypertension+vanadium treated domain (Fig. 1b).

3.3. Catalase activity in Kidney

Catalase activity was significantly lower in the vanadium treated domain (Fig. 2a). There was no significant difference between the control domain, hypertension and hypertension +vanadium treated domain (Fig. 2a)

3.4 Catalase activity in Liver

Catalase activity was significantly lower in the vanadium treated domain (Fig. 2b). There was no significant difference between control, vanadium treated and hypertension + vanadium treated domain (Fig. 2b).

3.5 Glutathione peroxidase activity in Kidney

Glutathione peroxidase activity was significantly lower in the vanadium treated domain and significantly higher in the hypertension induced domain (Fig. 3a). There was no significant difference between the control domain and hypertension +vanadium treated domain (Fig. 3a).

3.6. Glutathione peroxidase activity in Liver

Glutathione peroxidase activity was significantly lower in the vanadium treated domain and significantly higher in the hypertension domain (Fig. 3b). Glutathione peroxidase activity in hypertension+ vanadium treated domain was significantly lower than that of hypertension induced domain but significantly higher than that of control and vanadium treated domain (Fig. 3b).

3.7. Lipid peroxidation activity in Kidney

Lipid peroxidase activity was significantly lower in the hypertension induced domain and significantly higher in the vanadium treated domain (Fig. 4a). There was no significant difference between control domain and the hypertension + vanadium treated domain (Fig. 4a).

3.8. Lipid peroxidase activity in Liver

Lipid peroxidase activity was significantly higher in the vanadium treated domain and significantly lower in the hypertension induced domain than any other domain (Fig. 4b). There was no significant difference between the control and the hypertension + vanadium treated domain (Fig. 4b).

4. DISCUSSION

Although there are many reports on the activity of antioxidant enzymes in hypertension induced rats and in different animals [20-27]. This study deals with the treatment of hypertension with vanadium and effect of vanadium on antioxidant enzymes activities in major target organs of the body i.e., Kidney and Liver.

SOD activity showed a marked decrease in the kidney tissues of hypertension induced domain (Fig. 1a) which indicates that oxidative stress induced by a high fructose diet suppressed the activity of SOD. This observation in our study correlates with previous reports on SOD activity under oxidative stress and hypertension [24, 26]. But the vanadium treatment significantly increased the activity of SOD in renal tissues compared to that of hypertension induced domain (Fig. 1a). This marked increase in our study was similar to that of previous reports [21, 25, 27, and 28]. Vanadyl sulfate increased the activity [28]. But ammonium monovanate reduced the SOD activity [23]. Sodium metavanadate didn't cause any significant change in SOD activity [22].

In the case of hepatic tissues also hypertension induction greatly reduced the SOD activity (Fig. 1b). There were no previous reports on SOD activity in the rat liver in the hypertension induced rats. Whereas vanadium treatment acted effectively in increasing the SOD activity (Fig. 1b) and this increase in the activity compensated the effect of fructose induced reduction in the activity of SOD in the liver. Similar observations were observed in the previous reports [21, 25].

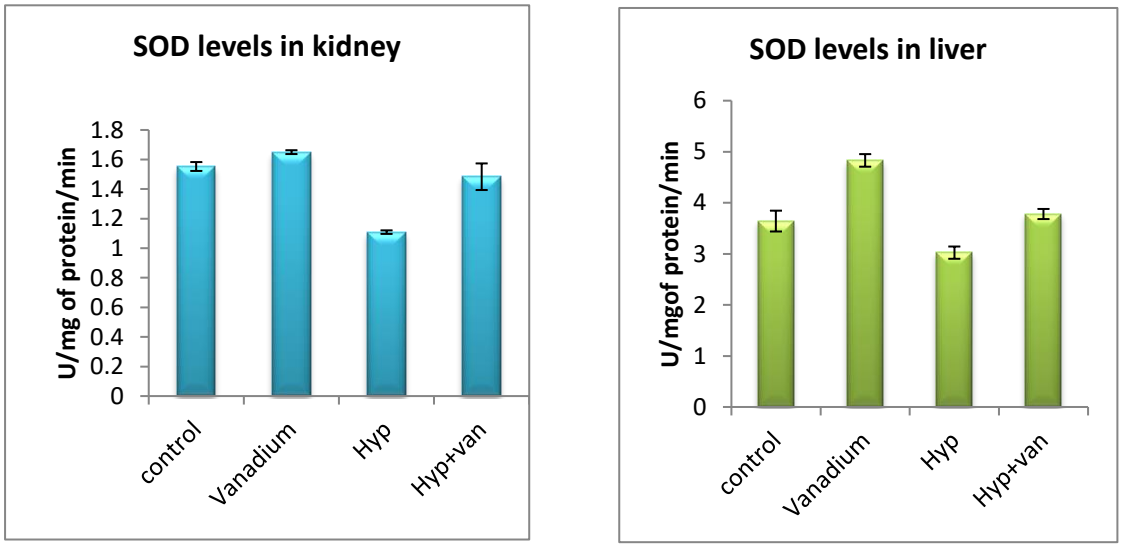
Catalase activity showed marked increase in the renal tissues of the hypertension induced domain (Fig. 2a) which indicates that oxidative stress induced by a high fructose diet stimulated the activity of catalase. This observation in our study correlates with previous reports on catalase activity under oxidative stress and hypertension. [24, 26]. But the vanadium treatment significantly decreased the activity of catalase in renal tissues compared to that of hypertension induced domain (Fig. 2b). This marked decrease in our study was similar to that of previous reports. [25, 27]. In case of hepatic tissue also hypertension induction markedly increased the catalase activity. Whereas vanadium treatment

acted effectively in decreasing the catalase activity and this decrease in the activity compensated the effect of fructose induced reduction in the activity of catalase. Similar observations were reported in the studies by Saxena *et al*. [21] and Genet *et al*. [25]

Glutathione peroxide activity showed a marked increase in the renal tissues of hypertension induced domain (Fig. 3a) which indicates that oxidative stress induced by a high fructose diet stimulated the activity of Glutathione peroxide. This observation in our study correlates with previous reports on Glutathione peroxide activity under oxidative stress and hypertension [24, 26]. But the vanadium treatment significantly decreased the activity of Glutathione peroxide in renal tissues compared to that of hypertension induced domain (Fig. 3b). This marked decrease in our study was similar to that of previous reports [25, 27]. Sodium metavanadate didn't cause any significant change in SOD activity [22].

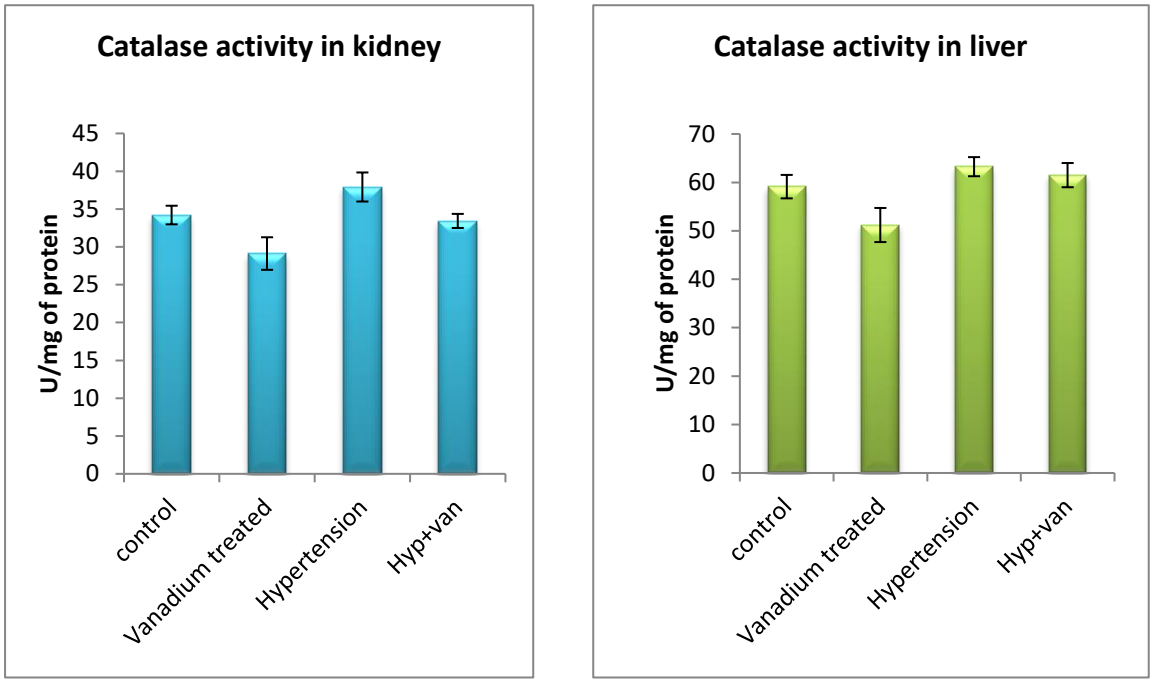
Hypertension induction markedly increased the Glutathione peroxide activity. Whereas vanadium treatment acted effectively in decreasing the Glutathione peroxide activity and this decrease in the activity compensated the effect of fructose induced reduction in the activity of liver glutathione peroxide. In contrast to our study the Glutathione peroxide levels were reported to be increased by Saxena *et al.* [21] and Tas *et al*. [28]

Lipid peroxidation activity showed a marked decrease in the kidney tissues of hypertension induced domain (Fig. 4a) which indicates that oxidative stress induced by high fructose diet suppressed the activity of Lipid peroxidation. This observation in our study correlates with previous reports on Lipid peroxidation activity under oxidative stress and hypertension [24, 26]. But the vanadium treatment significantly increased the activity of Lipid peroxidation in renal tissues compared to that of hypertension induced domain (Fig. 4b). Similar observations were reported by Donaldson *et al*. [29]. In the case of hepatic tissues also hypertension induction greatly reduced the Lipid peroxidation activity. Whereas vanadium treatment acted effectively in increasing the Lipid peroxidation activity and this increase in the activity compensated the effect of fructose induced reduction in the activity of Lipid peroxidation in liver.



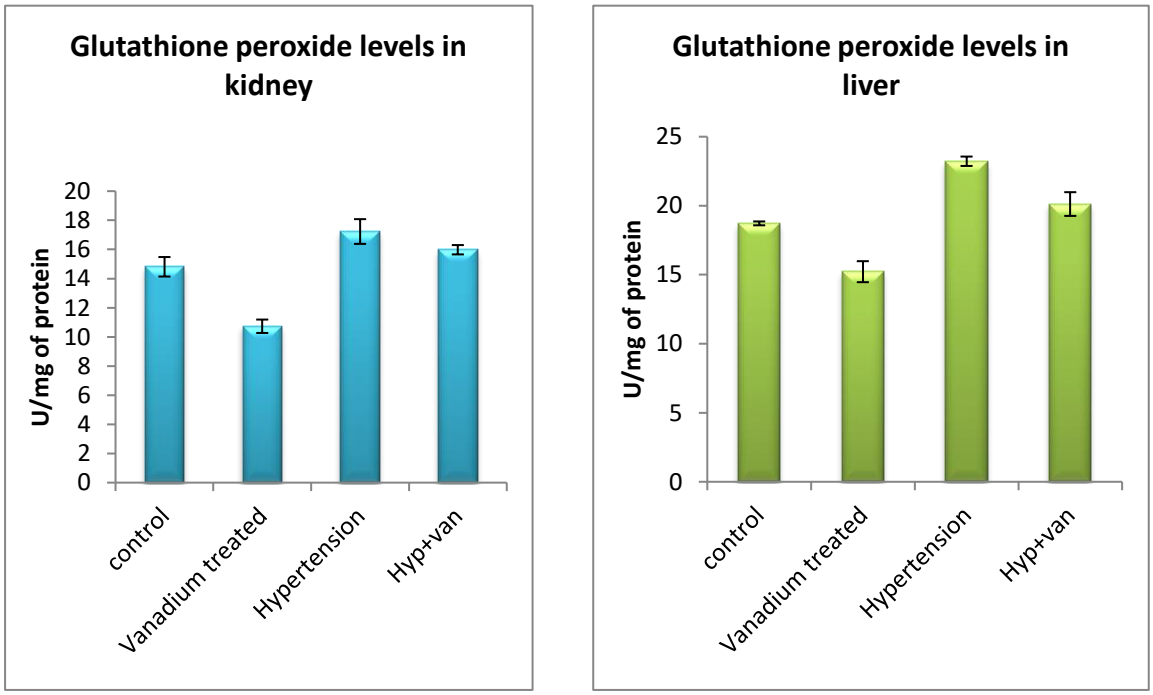
(a) (b)

Fig.1: SOD levels in kidney (a) and liver (b) of control, vanadium treated, Hypertension induced and hypertension induced+ vanadium treated domains of rats. The graph indicates that domain of control when compared with treated are not significantly different (P≤ 0.05).



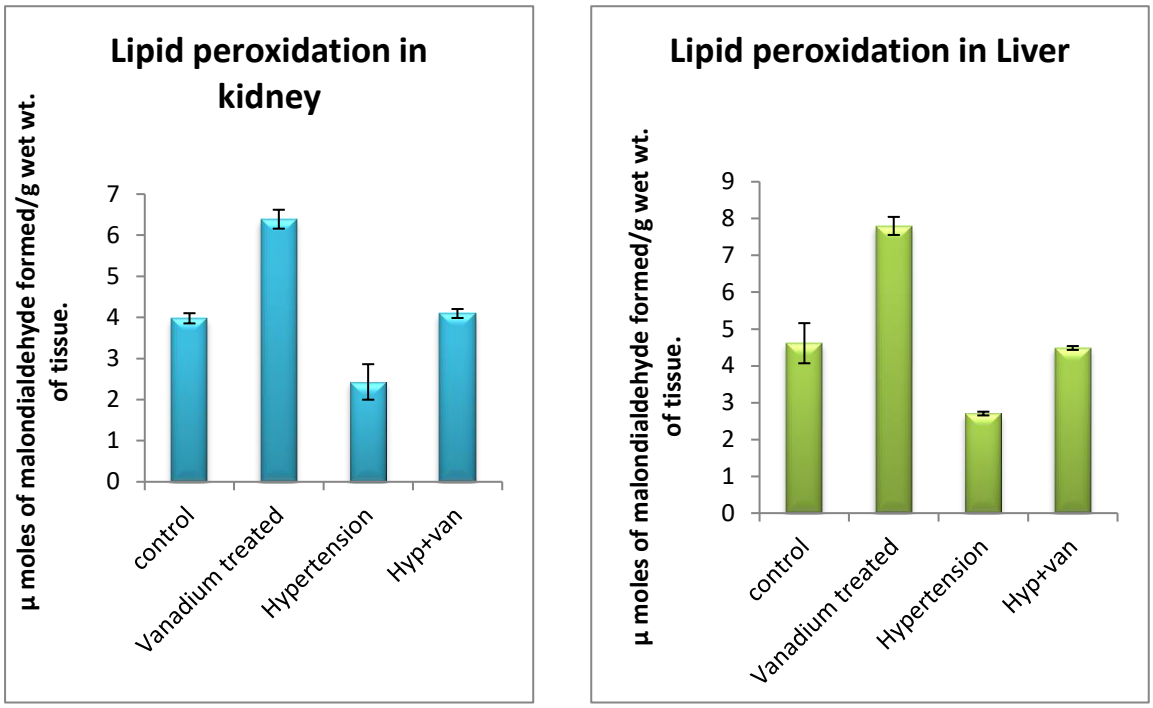
(a) (b)

Fig. 2: Catalase activity in kidney (a) and liver (b) of control, vanadium treated, hypertension induced and hypertension induced +vanadium treated domains of rats. The graph indicates that domain of control when compared with treated are not significantly different (P≤ 0.05)



(a) (b)

Fig. 3: Glutathione peroxidase levels in kidney (a) and liver (b) of control, vanadium treated, Hypertension induced and hypertension induced + vanadium treated domains of rats. The graph indicates that domain of control when compared with treated are not significantly different (P≤ 0.05).



(a) (b)

Fig.4: Lipid peroxidation levels in kidney (a) and liver (b) of control, vanadium treated, Hypertension induced and hypertension induced + vanadium treated domains of rats. The graph indicates that domain of control when compared with treated are not significantly different (P≤ 0.05).

5. CONCLUSION:

Thus from these studies, it is concluded that fructose induced hypertension altered the antioxidant enzyme activities and treatment with sodium metvanadate successfully restored the activity of all these antioxidant enzymes.

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