Original Communication

Vitamin C improves liver and renal functions in hypothyroid rats by reducing tissue oxidative injury

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Abstract: *Background:* The effects of Vit C on liver and renal function and the tissues oxidative damage was investigated in hypothyroid rats. *Materials and methods:* The pregnant rats were divided into 5 groups (n=6): (1) Control; (2) Propylthiouracil (PTU; 0.005%), (3–5) PTU plus 10, 100 or 500 mg/kg b.w. Vit C. The drugs were added to the drinking water of the dams and their pups during lactation period and then continued for the offspring through the first 8 weeks of their life. Finally, 7 male offspring from each group were randomly selected.*Results:* Thyroxine, protein and albumin concentrations in the serum and thiol content and superoxide dismutase (SOD) and catalase (CAT) activities in renal and liver tissues of hypothyroid group was lower (all $P<0.001$) while, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALK-P), creatinine and blood urea nitrogen (BUN) concentrations in the serum and malondialdehyde (MDA) in the liver and renal tissues were higher than the control (all $P<0.001$). All doses of Vit C increased thyroxine, protein and albumin and thiol content in in renal and liver tissues while, decreased AST, ALT and ALK-P concentration and MDA in liver and renal tissues compared to PTU group ($P<0.05$–$P<0.001$). Creatinine, BUN and SOD and CAT were improved by both 100 and 500 mg/kg of Vit C in the renal ($P<0.05$–$P<0.001$) and by 100 mg/kg in the liver ($P<0.05$–$P<0.001$).*Conclusion:* Vit C improved liver and renal function of hypothyroid rats which might be due to its protective effects against tissues oxidative damage.

Keywords: Vitamin C, hypothyroidism, liver, renal function, oxidative stress

Introduction

A change in thyroid function has been reported in patients with various spectra of liver diseases [1]. Also, thyroid dysfunction has been suggested to occur in patients with chronic kidney disease (CKD) [2]. Thyroid hormones are suggested to be able to crucially regulate physiological actions of the kidneys including glomerular filtration rate (GFR), renal blood flow and secretion and re-absorption of the molecules [3–6]. Some hemodynamic changes such as hyponatremia and a diminished level of renal blood and plasma stream are suggested to be accompanied with hypothyroidism status, which may affect renal capacity and diminish the GFR [7].

It is reported that thyroid hormones have antioxidant properties [8]. Moreover, an oxidative stress status has been reported to occur in hypothyroidism [9]. A significant modulation of various aspects of reactive oxygen species (ROS) metabolism [10] and antioxidant defenses in the liver by experimentally induced hypo- and hyperthyroidism has been suggested [11]. Also, hypothyroidism has been shown, which correlates with cirrhosis [12]. An abnormal thyroid hormone status is frequently linked to hepatic lipid homeostasis changes [13]. Other previous reports by

Korshunov et al. (1997) and Baskol et al. (2007) exhibited that hypothyroidism may induce ROS production [14, 15]. Also, it has been reported that oxidative stress in thyroid disorders has a correlation with CKD [16].

Propylthiouracil (PTU) is one of the most common drugs to treat hyperthyroidism [17]. However, liver tissues injury after administration of PTU has been reported [18]. Also, after adding PTU to drinking water of animals, alanine aminotransferase (ALT) [19] and aspartate aminotransferase (AST) values have been reported to be higher compared to the control [20]. An increased level of ALT and total bilirubin in the serum of PTU treated patients has also been reported [21]. Furthermore, histological analysis has revealed a hepatotoxicity status in PTU treated animals [22]. Administration of PTU has also been able to increase lipid peroxidation in the studied organs with a parallel decrease in antioxidants such as superoxide (SOD) and catalase (CAT) [23, 24].

Normal level of thyroid hormones has been well known to be vital for development of many organs [25]. Drugs that disrupt thyroid hormones during pregnancy may have an adverse effect on normal development [26]. Hypothyroidism during fetal or postnatal periods can lead to functional abnormalities in children [27]. In one study, it has been shown that hypothyroxinemia during pregnancy might be followed by liver function disorders in dams and offspring [28]. In this context, exposure to anti-thyroid drugs such as methimazole during fetal and neonatal periods of life induces a congenital neonatal hypothyroidism and influences the pattern of genes that are under the control of thyroid hormones during rat development [10]. In addition, disturbances in endocrine status during the neonatal period of life may affect the susceptibility to chronic diseases or biological insults in adulthood [29]. Researchers showed that even a transient neonatal hypothyroidism influences the transcriptional program of genes involved in lipid metabolism in the liver accompanying with a decreased level of the liver weight [30].

Vitamin (Vit) C is a naturally-occurring water-soluble antioxidant present in cells, body fluids, and plasma [31]. In addition to acting as a ROS scavenger [32], Vit C plays a role as an essential coenzyme in the oxidative stress pathways; also, interactions have been demonstrated between Vit C and proline hydroxylase, lysine hydroxylase, 4-hydroxy phenyl pyruvate dioxygenase, dopamine-hydroxylase, tryptophan hydroxylase, and γ-butyrobetaine hydroxylase [33]. Fipronil is a member of the phenylpyrazole class of pesticides, which is being extensively used in the agriculture and veterinary medicine [34]. Besides an increased level in antioxidant enzymes activities, Vit C has been able to prevent an increased level of lipid peroxidation induced by a high dose of fipronil [35]. Treatment with Vit C in methylmercury-exposed animals has led to a significant decrease in malondialdehyde (MDA) concentration and hepatic enzyme activities as well as a significant increase in the levels of glutathione (GSH) and total antioxidant capacity [36].

The objective of this study was to investigate the effects of administration of Vit C during neonatal and juvenile growth on liver and renal function in PTU-induced hypothyroid rats. Protective effects against tissues oxidative damage were also investigated as a possible mechanism.

Materials and methods

Drugs

PTU was purchased from the Sigma (Sigma Aldrich Chemical Co. St. Louis, MO). Other compounds, which were used for biochemical assessments, were purchased from the Merck Company (Darmstadt, Germany).

Animals and treatments

Thirty pregnant Wistar rats (12 weeks old and weighing 220–250 g) were purchased from the animal center of the Mashhad University of Medical Sciences, Mashhad, Iran, and kept in separate cages at 22 ± 2 °C in a room with a 12-hour light/dark cycle (lights on at 7:00 AM). Animal handling and all related procedures were carried out in accordance with the rules set by the Ethical Committee of the Mashhad University of Medical Sciences. After delivery, the mothers and their pups were randomly divided into five groups (n=6) and treated: (1) the Control group that received normal drinking water; (2) the Hypothyroid (Hypo) group that received PTU (0.005%, W/V) in their drinking water to develop hypotyhroidism; and (3–5) three groups including Hypo-Vit C 10, Hypo-Vit C 100 and Hypo-Vit C 500, which, besides PTU, received 10, 100 or 500 mg/kg Vit C [37–39]. During lactation period, the treatments were added to the drinking water of the mothers and their offspring. After lactation period, male offspring rats continued to receive the mentioned treatment through the first 8 weeks of their life. The offspring of each group was pooled and 7 male rats were randomly selected from each group.

Biochemical assessment

The animals were deeply anaesthetized using a high dose of urethane and the blood samples were collected to use for thyroxine assessment, renal function parameters and liver function tests. The rats were then sacrificed and the liver

and renal tissues were removed to analyze for MDA concentration, total thiol (SH) content, SOD and CAT [23]. The samples were stored in a freezer (−80 °C) until further use.

Liver and renal function tests

The serum samples were analyzed for creatinine and blood urea nitrogen (BUN) using commercial kits (Pars Azmoon Company, Tehran, Iran). AST, ALT, alkaline phosphatase (ALK-P), total protein and albumin were also measured by an automatic analyzer (Hitachi 902) using the kits and based on the manufacturer's instructions.

Liver and renal tissues oxidative damage criteria

MDA reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance (TBARS) to produce a red color. In brief, 2 ml of TBA/trichloroacetic acid (TCA)/ hydrochloricacid (HCL) reagent were added to 1 ml of tissue homogenates and the solution was incubated in a boiling water bath for 40 min. After cooling, the whole solution was centrifuged (1000 g for 10 min). The absorbance of the supernatant was measured at 535 nm. The MDA concentration was calculated using a formula, which has been previously described [40, 41].

Total thiol content was measured using DTNB (2, 2'-dinitro- 5, 5'-dithiodibenzoic acid), which reacts with the SH groups to produce a yellow color [42]. Briefly, 1 ml of tris-(ethylenediaminetetraacetic acid) EDTA buffer was added to 50 µl of the tissue homogenates and the absorbance was read at 412 nm against Tris-EDTA buffer. Then, 20 µl of DTNB reagent (10 mM) were added to the mixture and after 15 min incubation at room temperature, the absorbance was again read. The absorbance of the DTNB reagent was also read as a blank. Total thiol concentration was calculated based on an equation previously described [40, 41].

SOD activity was measured using a method described by Madesh and Balasu Bramanian [43]. A colorimetric assay involving the generation of superoxide by pyrogallol autooxidation and inhibition of superoxide-dependent decrease of the tetrazolium dye, MTT (3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide) to its formazan by SOD was measured at 570 nm. One unit of the SOD activity was characterized as the amount of enzyme causing 50% inhibition in the MTT reduction rate.

CAT activity was estimated using a method described by Aebi [44]. The principle of the assay is based on the determination of the rate constant, k, (dimension: s^{-1}, k) of hydrogen peroxide decomposition. By measuring the decrease in absorbance at 240 nm/min, the rate constant of the enzyme was measured. Activities were expressed as k (rate constant) per liter.

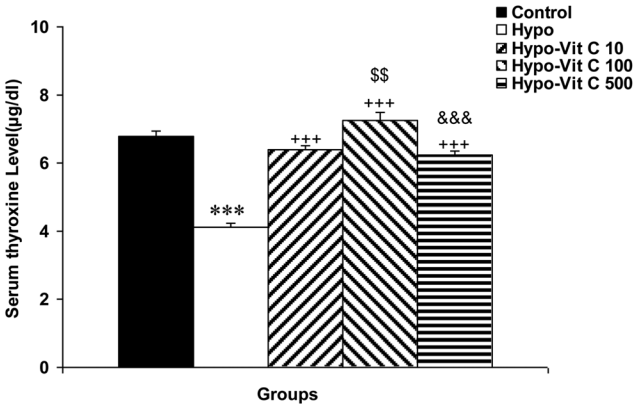

Figure 1. The effect of Vit C on thyroxine levels in PTU-induced hypothyroidism. Data are presented as mean±SEM (n=7 in each group). ***P<0.001 compared to the control group, $^{+++}$P<0.001 compared to the Hypo group, $^{\$\$}$P<0.01 compared to the Hypo- Vit C 10 group, $^{\&\&\&}$P<0.001 compared to the Hypo-Vit C 100 group. Hypo: Hypothyroidism, Vit C 10: Vit C 10 mg/kg, Vit C 100: Vit C 100 mg/kg and Vit C 500: Vit C 500 mg/kg.

Statistical analysis

All data were expressed as mean ± SEM. The normality of the data was tested using the Kolmogorov-Smirnov test. Differences in variance were tested using the Levene's test. All the data were compared by one-way ANOVA followed by Tukey's post hoc comparisons test. Differences were considered statistically significant when $p<0.05$.

Results

Serum thyroxine level

The results showed that the offspring of the animals of PTU treated rats had a lower serum concentration of thyroxine compared to the control (P<0.001). All the three doses of Vit C improved the thyroid glands function, which was reflected in an increased level of serum thyroxine in the serum of the animals treated by 10, 100 and 500 mg/kg of Vit C (P<0.001, as shown in Figure 1). The results also showed that the medium dose was more effective than the lowest (P<0.01) and the highest dose (P<0.001) (Figure 1)

Liver function criteria

The results of Vit C on liver function criteria of the hypothyroid rats are shown in Figure 2. In the hypothyroid rats, the

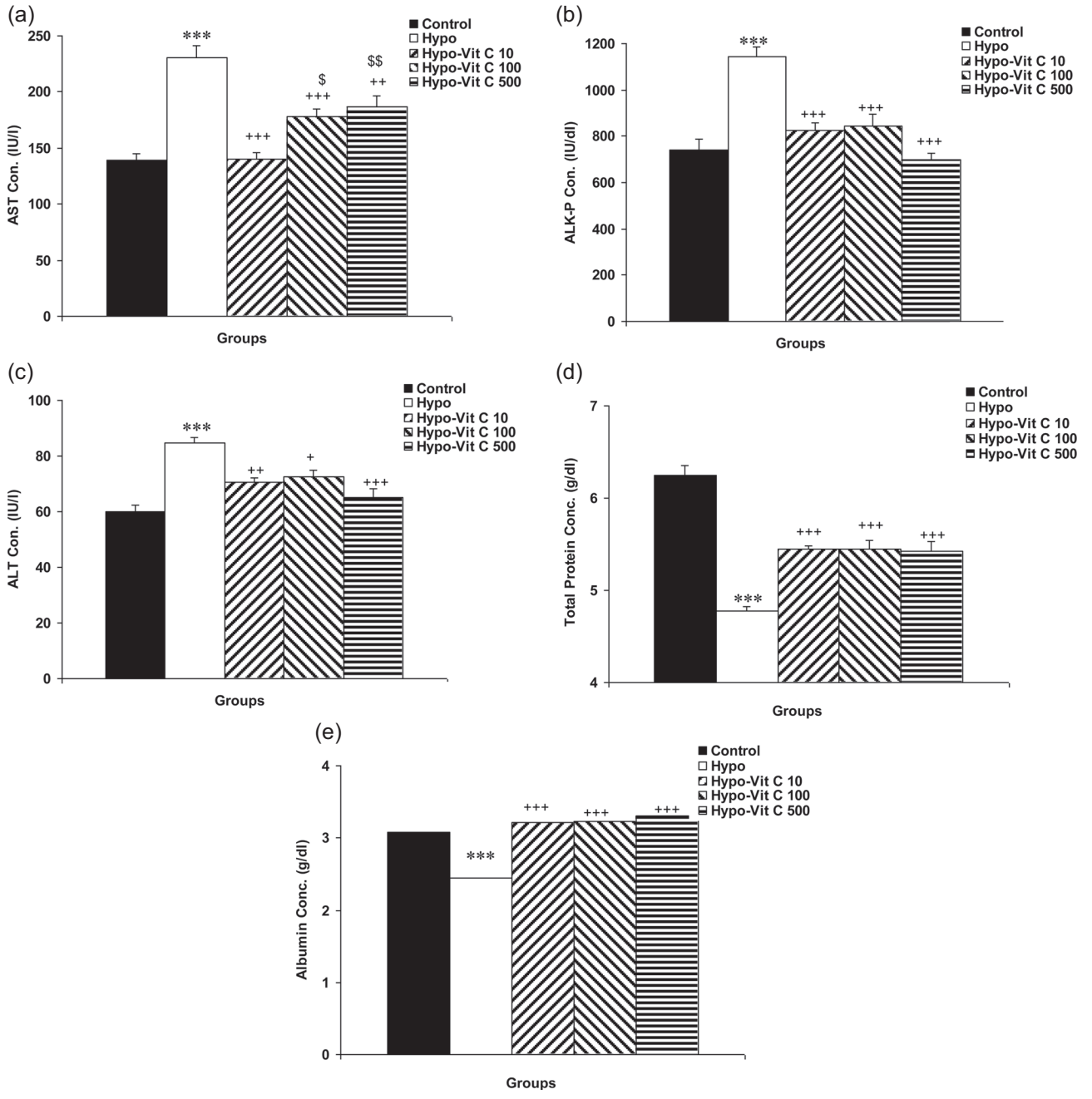

Figure 2. The effect of Vit C on serum AST (a), ALT (b), ALK-P (c), total protein (d) and albumin (e) concentrations in PTU-induced hypothyroidism. Data are presented as mean ± SEM (n=7 in each group). ***P<0.001 compared to the control group, $^{+++}$P<0.001 compared to the Hypo group, $^{\$}$P<0.05, $^{\$\$}$P<0.01 compared to the Hypo-Vit C 10 group. Hypo: Hypothyroidism, Vit C 10: Vit C 10 mg/kg, Vit C 100: Vit C 100 mg/kg and Vit C 500: Vit C 500 mg/kg.

serum AST concentration was higher than that in the control (P<0.001). Treatment of the animals by all the three doses including 10, 100 and 500 mg/kg of Vit C attenuated the serum concentration of AST in a dose dependent manner (P<0.001, P<0.001, and P<0.01, respectively). The results also showed that the two higher doses including 100 and 500 mg/kg of Vit C were more effective than the lowest dose (P<0.05 and P<0.01, respectively) (Figure 2a). Hypothyroidism status also increased the serum ALT compared to the control group (P<0.001). Similar to AST, all the three doses of Vit C prevented any increase in ALT concentration due to hypothyroidism conditions (P<0.01 for 10 mg/kg, P<0.05 for 100 mg/kg and P<0.001 for 500 mg/kg). However, there were no significant differences between the effects of the three doses of Vit C on the ALT concentration (Figure 2b). Furthermore, the

lowering effects of hypothyroidism induced by PTU on liver function was confirmed when it was seen that the serum ALK- P concentration in the PTU exposed rats was significantly higher than that in the control ones (P<0.001). Co-treatment by 10 mg/kg (P<0.001), 100 mg/kg (P<0.001) and 500 mg/kg (P<0.001) Vit C attenuated the serum ALK-P concentration compared to the PTU group. Also, there were no significant differences between the effects of the three doses of Vit C (Figure 2c).

The results showed that hypothyroidism status lowered both total protein and albumin compared to the control group (P<0.001, as in Figures 2d & e). The results also revealed that all the three doses of Vit C increased the total protein concentration compared to the PTU group (P<0.001 for all, as in Figure 2d). Additionally, the serum albumin concentration in the rats treated by all the doses of Vit C was higher than that in the PTU group (P<0.001 for all, according to Figure 2e).

Liver tissues oxidative damage criteria

The liver MDA concentration of the hypothyroid group was significantly higher than that of the control group (P<0.001). In addition, all the three doses of Vit C had a lower concentration of MDA in the liver tissues of the rats that received 10–500 mg/kg of Vit C during neonatal and juvenile periods (P<0.001 for 10 and 100 mg/kg and P<0.01 for 500 mg/kg compared to the PTU group) (Figure 3a). Additionally, the highest dose was more effective than both the medium and lowest doses (P<0.05 and P<0.001) (Figure 3a). Administrating PTU during lactation period and continuing up to the first 8 weeks of the life of the pups attenuated the liver tissues thiol contents (P<0.001). Vit C administration improved the thiol contents of the liver tissues (P<0.001 for all the doses of Vit C compared to the PTU group). The results also showed that the medium dose of Vit C was more effective than both its lowest and highest doses (P<0.01 for the both) (Figure 3b). A comparison of the SOD activity in the liver tissues showed a significant lower level in the hypothyroid than the control group (P<0.01). Only a medium dose of Vit C was effective to enhance the SOD activity in the liver tissues compared to the PTU group (P< 0.05). Neither 10 nor 500 mg/kg of Vit C had a significant effect of the SOD activity in the liver tissues compared to the PTU group (Figure 3c). As shown by Figure 3c, the medium dose was more effective than the highest dose (P<0.01). It was also observed that the CAT activity in the liver tissues of the hypothyroid group was significantly lower that of the control group (P<0.001). The findings also showed that the medium dose of Vit C increased the CAT activity in the liver tissues compared to the hypothyroid group (P<0.001). No significant difference was observed between the rats treated with 10 and 500 mg/kg of Vit C compared to the PTU group. Additionally, the medium dose was more effective than the highest dose (P<0.01) (Figure 3d).

Renal function criteria

The results showed that hypothyroidism induced by PTU during neonatal and juvenile period affected renal function of the exposed rats. BUN concentration in the serum of the hypothyroid group was higher than that in the control group (P<0.001). Treatment by Vit C improved renal function of the hypothyroid rats, presented by a lower level of BUN in the serum of the animals in the groups treated by 100 and 500 mg/kg of Vit C compared to the PTU group (P<0.001); however, 10 mg/kg of Vit C was not effective (Figure 4a). The results also showed that serum BUN concentration in the animals of the Hypo-Vit C 100 and Hypo-Vit C 500 groups was lower than that in the PTU-Vit C 10 group (P<001) (Figure 4a).

Similar to BUN, PTU administration increased serum creatinine in the hypothyroid group compared to the control group (P<0.001). Protective effects of Vit C on renal function was confirmed when the serum creatinine level was compared between the groups. The results showed that the two higher doses including 100 and 500 mg/kg of Vit C attenuated the serum creatinine concentration compared to PTU group (P<0.001 and P<0.01, respectively). The lowest dose of Vit C was not able to change the serum concentration of creatinine. The results also showed that the two higher doses including 100 and 500 mg/kg of Vit C were more effective than the lowest dose (P<0.001 and P <0.01, respectively) (Figure 4b).

Renal tissues oxidative damage criteria

Renal tissue MDA in the hypothyroid group was higher than that in the control group (P<0.001). Pretreatment of the animals by 10, 100 and 500 mg/kg of Vit C brought about a diminished level of MDA in the renal tissues compared to the hypothyroid group (P<0.01–P<0.001) (Figure 5a). The results showed that the medium dose of Vit C was more effective than both the lowest (P<0.001) and highest doses (P<0.05).

Hypothyroidism status also lessened the thiol content in the renal tissues (P<0.001) in comparison to the control group. Treatment of the animals by all the three doses including 10, 100 and 500 mg/kg of Vit C significantly increased levels of total thiol in the renal tissues (P<0.05–P<0.001) (Figure 5b). The two higher doses including 100 and 500 mg/kg of Vit C were more effective than the lowest dose (P<0.001). The SOD activity in the renal tissues of the hypothyroid group was lower than that in the control group

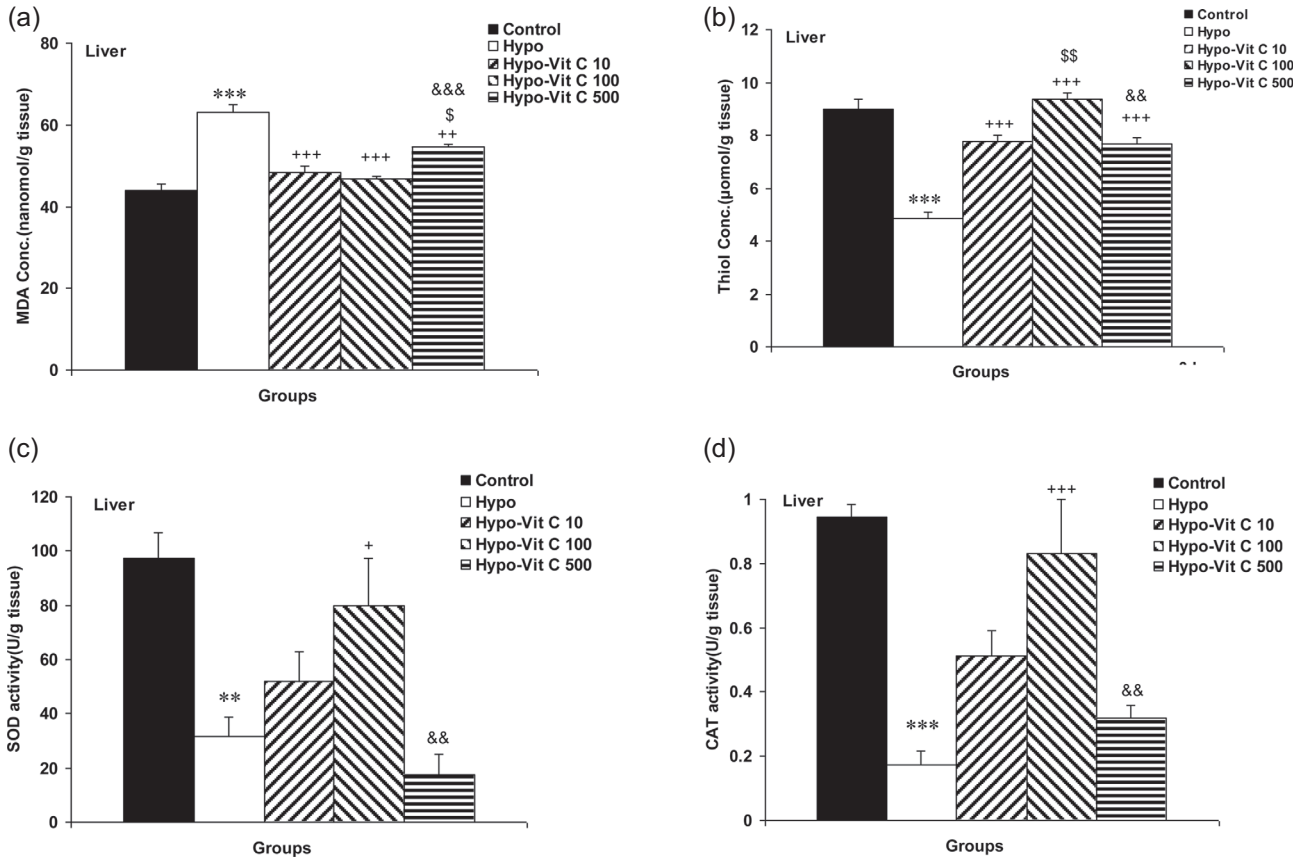

Figure 3. The effect of Vit C on liver tissues MDA (a) and total thiol (b) concentrations as well as SOD (c) and CAT (d) activities in PTU-induced hypothyroidism. Data are presented as mean ± SEM (n=7 in each group). **P<0.01 and ***P<0.001 compared to the control group, $^{+}$P<0.05, $^{++}$P<0.01 and $^{+++}$P<0.001 compared to the Hypo group, $^{\$}$P<0.05 and $^{\$\$}$P<0.01 compared to the Hypo-Vit C 10 group, $^{\&\&}$P<0.01 and $^{\&\&\&}$P<0.001 compared to the Hypo-Vit C 100 group. Hypo: Hypothyroidism, Vit C 10: Vit C 10 mg/kg, Vit C 100: Vit C 100 mg/kg and Vit C 500: Vit C 500 mg/kg.

(P<0.001). The animals in the PTU-Vit C 100 and PTU-Vit C 500 groups demonstrated an increment in the SOD activity in the renal tissues compared to the hypothyroid group (P<0.01 and P<0.05, respectively) (Figure 5c). Additionally, the two higher doses were more effective than the lowest dose (P<0.01 and P<0.05, respectively). The CAT activity of the renal tissues in the hypothyroid group was lower, as compared to the control group (P<0.001). Also, the animals treated by 100 and 500 mg/kg of Vit C demonstrated an increased level of the CAT action in the renal tissues compared to the hypothyroid group (P<0.001). Additionally, both 100 and 500 mg/kg of Vit C were more effective than 10 mg/kg of Vit C to improve the CAT activity in the renal tissues (Figure 5c).

Discussion

Thyroid hormones have been well documented to affect the functions of nearly all organs and cells in the body [45]. The data presented here clearly indicates how biochemical markers of liver and kidney may be affected by alteration in the level of thyroid hormones in the body. The current study demonstrated that PTU exposure during neonatal and juvenile period was resulted in development of hypothyroidism, and negatively influenced liver and renal function in rats. In the current study, PTU decreased serum thyroxine of offspring to a level, which has been reported to be seen in overt hypothyroidism status [46].

It is suggested that both hyperthyroidism and hypothyroidism states affect liver function tests. For example, an increased level of plasma concentration of total bilirubin and the liver enzyme activities in both hyperthyroid and hypothyroid subjects have been reported [47]. Enzymatic activities of AST and ALT are sensitive serological indicators of liver function. Higher activities of these enzymes in the serum have been found in response to oxidative stress induced by hyperthyroidism [48]. Normal circulating levels of thyroid hormones are required for both normal hepatic circulation and normal bilirubin metabolism [49].

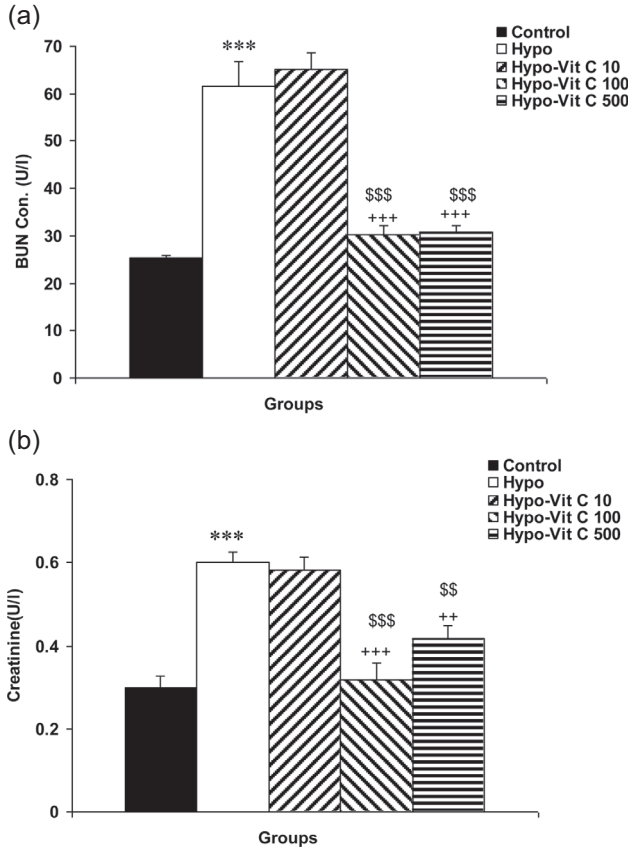

Figure 4. The effect of Vit C on serum BUN (a) and creatinine (b) concentrations in PTU- induced hypothyroidism. Data are presented as mean±SEM (n=7 in each group). ***P<0.001 compared to the control group, $^{++}$P<0.01 and $^{+++}$P<0.001 compared to the Hypo group, $^{\$\$}$P<0.01 and $^{\$\$\$}$P<0.001 compared to the Hypo-Vit C 10 group, $^{\&\&}$P<0.01 and $^{\&\&\&}$P<0.001 compared to the Hypo- Vit C 100 group. Hypo: Hypothyroidism, Vit C 10: Vit C 10 mg/kg, Vit C 100: Vit C 100 mg/kg and Vit C 500: Vit C 500 mg/kg.

In this study, we showed that serum AST, ALT and ALK-P concentrations in the hypothyroid group were higher than in the control group. Overall, our findings confirm the previous observation suggesting that primary hypothyroidism is associated with an elevated level of serum liver enzyme concentrations [50]. The liver enzymes including ALT and AST have been shown to have a significant positive correlation with serum thyroid stimulating hormone (TSH) levels and a negative correlation with serum T4 levels [51].

Serum total protein always represents the excretory and synthetic functions of liver [28]. In this study, serum total protein and albumin of the hypothyroid group was lower than that of the control group. In agreement with our results, Patel et al. (2013) also showed that the serum total protein, albumin and globulin in hypothyroidism were low compared to the control group [52].

On the other hand, Verghes et al. (2010) reported that SOD activity in the liver tissues of hypothyroidism induced by PTU in rats was low, indicating that hypothyroidism induces an oxidative damage in liver tissues [53]. In the present study, the MDA concentration of the liver tissues in the hypothyroid group was high, while thiol, CAT and SOD in the liver tissues were low compared to the control animals. These results confirmed an oxidative stress status in the liver tissues due to PTU-induced hypothyroidism. Accordingly, in another study, an increased level of hepatic lipid peroxidation in PTU treated animals has been reported [54]. However, in contrast with our study, it has been previously shown that PTU-induced hypothyroidism reduces oxidative damage in the lung, hepatic and renal tissues, probably due to hypo metabolism, which is associated with a decreased production of reactive oxygen metabolites and enhancement of antioxidant mechanisms [55].

A relationship between the levels of thyroid hormones and the physiological actions of the kidneys has been suggested [3–6]. In addition, an increased level of creatinine in the serum of hypothyroid patients has been reported [56]. Also, a relationship between hypothyroidism and kidney dysfunction has been suggested [57, 58]. Our study also implies that reduction of thyroidal hormones affected the kidney function in rats, which was reflected by an increased level of both creatinine and BUN as markers of GFR. Consistent with the results of the present study, Den Hollander et al. (2005) observed an elevated level of serum creatinine in hypothyroid patients [3]. It has also been reported that hypothyroidism can cause reductions in GFR; thus, a screening for hypothyroidism in patients with unexplained elevations in serum creatinine has been suggested [59]. Our results also showed that the rats affected by hypothyroidism revealed a decreased level in total thiol concentration, CAT, and SOD activities, while an increased level of MDA concentration in the renal tissues. In line with the result of our study, Baltaci et al. (2014) demonstrated that the renal tissues MDA increased in an experimental hypothyroidism model induced by 4-weeks PTU administration, while the levels of GSH decreased [19]. Considering these results, tissues oxidative damage as a possible mechanism for deleterious effects of hypothyroidism on renal and liver functions might be suggested.

Vit C, known as L-ascorbic acid, is a naturally existent organic substance marked by antioxidant property, and is also an essential nutrient for humans [60]. Vit C has been well known as an electron donor and as an essential cofactor for biosynthesis of intracellular biochemicals. Once avitaminosis occurs, the person suffers from severe scurvy symptoms [61]. As an antioxidant agent, Vit C has been reported to play an important protective role against insecticide-induced hepatic toxicity and to prevent the effect of free radicals on vital cells [62]. It was shown that Vit C level is lower in pregnant women with type 1 diabetes [63]. In the present study, we showed that treatment with all the doses

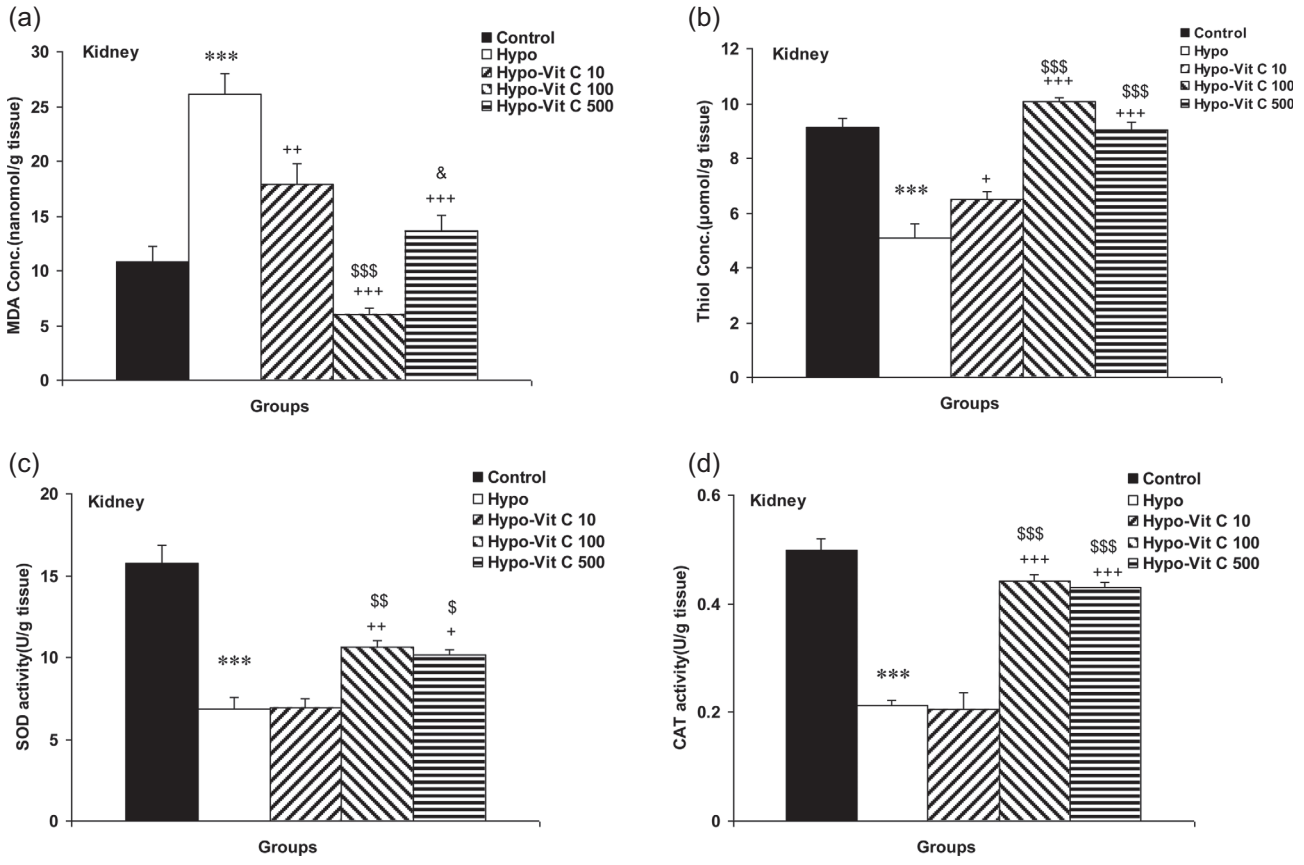

Figure 5. The effect of Vit C on renal tissues MDA (a) and total thiol (b) concentrations as well as SOD (c) and CAT (d) activities in PTU-induced hypothyroidism. Data are presented as mean±SEM (n=7 in each group). ***P<0.001 compared to the control group, $^{+}$P<0.05, $^{++}$P<0.01 and $^{+++}$P<0.001 compared to the Hypo group, $^{\$}$P<0.05, $^{\$\$}$P<0.01 and $^{\$\$\$}$P<0.001 compared to the Hypo-Vit C 10 group, $^{\&}$P<0.05 compared to the Hypo-Vit C 100 group. Hypo: Hypothyroidism, Vit C 10: Vit C 10 mg/kg, Vit C 100: Vit C 100 mg/kg and Vit C 500: Vit C 500 mg/kg.

of Vit C decreased AST, ALT and ALK-P compared with the hypothyroid group. The results also showed that Vit C was able to improve both total protein and albumin in the serum. The other researchers also showed that Vit C had a potent protective effect against diazinon-induced hepatotoxicity in rats, which was reflected in a significant reduction in ALT and AST activities compared to the diazinon group. The beneficial effects were attributed to the scavenging of free radicals and increasing of antioxidant status [64]. Oral administration of Vit C has been reported to be able to reduce AST, ALT and ALK-P activities in the serum of lead exposed rats [65]. It has also been demonstrated that Vit C can reduce malathion-induced hepatotoxicity in rats [66]. In consistent with our study in which Vit C administration significantly increased serum concentrations of albumin and total-protein, Liang et al. also showed that these parameters decreased in a liver injury model induced by Concanavalin A in mice [67]. Vit C was administered at doses that had been previously used to protect liver injury [68]. Considering the results of the present study, the effects of Vit C on AST seems to be dose dependent. However, there were no significant differences on the effects of three doses of Vit C on other functional tests of the liver. Additionally, the medium dose of Vit C on liver tissues oxidative damage criteria was more effective than the low and high doses. Considering these results, a pro-oxidant effect for high doses of Vit C might be suggested, while Vit C acts as an anti-oxidant agent when administered at lower doses [69, 70]. In addition, besides protective effects against liver tissues oxidative damage, other mechanism(s) such as anti-inflammatory effects may also have a role in the beneficial effects of Vit C, as seen in the present study, and are suggested to be evaluated in the future.

The results of the present study showed that Vit C was able to improve renal function of the hypothyroid rats, which was reflected in a decreased level of creatinine and BUN. Similarly, Vit C has been reported to prevent oxidative stress in end-stage renal disease with scavenging free radicals [23]. The therapeutic effect of Vit C has been repeatedly attributed to its anti-oxidant properties. In the current study, treatment by all the doses including 10, 100 and 500 mg/kg of Vit C reduced the MDA concentration, while

increased thiol content in the liver tissues. Both SOD and CAT activities in the liver tissues of the rats treated by the medium dose of Vit C increased; however, the highest and lowest doses were not effective. Vit C has also been reported to be able to attenuate serum MDA concentration in depressed rotational workers [71]. It has been previously reported that high intake of Vit C exhibited a pro-oxidant activity that was associated with the production of the anion radical superoxide (O2−) [68]. Similarly, supplementation of Vit C in high amount had adverse effects including diarrhea and gastrointestinal disturbances in most adults [72].

In this study, Vit C was able to increase total thiol concentration, CAT, and SOD activities in the renal tissues, while it decreased MDA. It is suggested that Vit C may have protective effects on renal functions because of its antioxidant effects, which has also been reported in other studies [73, 74].

This study showed that Vit C significantly increased serum T4 level. It has been already reported that Vit C significantly increases the concentration of T4, T3 and decreases the TSH [75]. Therefore, the balancing effects on thyroid hormones can be suggested as one of the possible mechanisms, which indirectly contribute to effects of Vit C on improving liver and renal function in the present study. In supporting this idea, it has been reported that Vit C protects the thyroid gland of rats from damages induced by other toxicants, while increases serum TSH, T3 and T4 concentrations [76]. Thus, it could be suggested that the improvement effect of Vit C could be partially because of an antioxidant defense system that may protect the gland against PTU toxicity. The exact mechanism(s) responsible for improving effects of Vit C on thyroid functions needs to be further investigated in future studies. Furthermore, more investigations are needed to clarify the exact mechanism(s) involved in the liver and renal protective effects of Vit C. Therefore, more precise further studies using other animal models of hypothyroidism such as thyroidectomy are suggested to be carried out to better understand the mechanism(s).

Meanwhile, the results of the present study showed that a diet supplemented with Vit C improved the liver and kidney functions of the hypothyroid rats during neonatal and juvenile growth. Considering these results, it seems that medium doses of Vit C are more effective than high doses; however; it needs to be further investigated. Additionally, further molecular studies are suggested to better understand the exact mechanism(s).

In conclusion, the results of this study demonstrated that Vit C improves the renal and liver functions of the rats, which were subjected to a hypothyroidism status during neonatal and juvenile growth. It is suggested that the effects of Vit C are due to its protective effects against tissues oxidative damage.

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Conflicts of interests
The authors declare that there are no conflicts of interest.

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