ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 7, Suppl 1, 2014

ISSN - 0974-2441

Research Article

AMELIORATING EFFECTS OF PYRROLOQUINOLINE QUINONE (PQQ) ON PTU INDUCED OXIDATIVE DAMAGE IN MICE KIDNEY

NARENDRA KUMAR*[1], ANAND KAR[2]

[1, 2]**Thyroid research unit, School of Life Sciences, Devi Ahilya University, Takshashila Campus, Khandwa Road- 452017, M.P., India.**
Email: narendrakumar93@gmail.com

Received: 15 December 2013, Revised and Accepted: 12 January 2013

ABSTRACT

Objective: The present study undertaken to investigate the hitherto unknown potential of pyrroloquinoline quinone (PQQ) has been evaluated in ameliorating PTU induced oxidative damage in adult mice kidney.
Material and Method: Five groups of 7 each healthy male mice were established. Group I animals receiving simple drinking water served as control, whereas those of group II, III, IV and V received either PTU (0.05% in drinking water) alone for 5 weeks or PTU + three different concentrations of PQQ (1, 5 and 10 mg/kg/d, i.p. for 6 days), after which alterations in tissue lipid peroxidation (LPO) and in enzymatic activities of superoxide dismutase (SOD) and catalase (CAT) and in glutathione (GSH) content were evaluated in kidney. Simultaneously, concentrations of serum glucose, total cholesterol, glutamate oxaloacetate transaminase (SGOT), creatinine and urea were measured in serum.
Result: PTU administration enhanced the tissue LPO, serum SGOT, total cholesterol, creatinine and urea with a parallel decrease in serum glucose and tissue antioxidants such as SOD and CAT. However, when PTU treated animals received PQQ; these adverse effects were ameliorated, as it reduced the LPO, with a parallel increase in cellular antioxidants.
Conclusion: Out of three different doses of PQQ (1, 5 and 10 mg/kg), 10 mg/kg body weight was found to be the most effective and antiperoxidative in nature; Findings from this study revealed for the first time, that PQQ has the potential to ameliorate PTU-induced oxidative damage in kidney of mice, indicating the possible beneficial effect of the test compound in regulating PTU induced oxidative damages.

Keywords: PQQ, PTU, Kidney, Antioxidant, SGOT, Mice

INTRODUCTION

Pyrroloquinoline quinone (PQQ), an anionic, water soluble compound was initially isolated from cultures of methylotropic bacteria as a crystalline acetone adduct and was proposed to be a cofactor of many bacterial primary alcohol dehydrogenases, methanol dehydrogenase, glucose dehydrogenase and aldehyde dehydrogenase [1-4]. This compound has been attributed with multiple physiological functions such as regulation of electron transport system [5] enhancing the adaptability of microbes [6], improving the growth of plants [7] and stimulating the production of nerve growth factor [8], thus presenting a wide application prospect in pharmaceutical, agriculture and food industries [9-11].

Recently, it has been reported that PQQ scavenges reactive oxygen species (ROS) [12,13] and protects cells from oxidative stress-induced damage, effectively improves the activities of free radical scavenging enzymes and decreases the levels of free radicals as well as lipid peroxidation (LPO) [14-16]. Furthermore, PQQ prevents oxidative stress-induced neurotoxicity and neuronal death [17-20]. Both *in vivo* and *in vitro* studies have shown that PQQ can protect against several types of oxidative damage and toxic injury, as well as stroke damage and irradiation injury [21-27]. Despite all these beneficial actions of PQQ, no attempt was made so far by any worker to evaluate its role in the regulation on the oxidative damage induced by PTU/ hypothyroidic conditions.

Thyroid hormones are involved in the regulation of numerous body functions including lipid and carbohydrate metabolism, oxygen consumption and several physiological functions such as development, reproduction and growth [28,29]. Alterations in their normal levels cause some biochemical and clinical abnormalities such as hypothyroidism. Further, thyroid dysfunction is postulated to be closely related to ROS formation, which might account for thyroid hormone-induced tissue damage. It is also known that thyroid dysfunction increases LPO reactions and ROS [30,31]. LPO is an autocatalytic mechanism leading to oxidative destruction of cellular membranes [32]. Such destruction can lead to cell death and to the production of toxic and reactive aldehyde metabolites called free radicals, where malondialdehyde (MDA) is the most important product. It is further known that ROS lead to the oxidative damage of biological macromolecules, including lipids, proteins and DNA [33].

Since PQQ is known to regulate oxidative damage [30,34] and hypothyroidism is associated with oxidative stress, it was thought that the compound may ameliorate PTU induced oxidative damage in kidney. It is a well-known fact that kidney is involved in the regulation, metabolism and elimination of thyroid hormone (TH) and is also an important target organ for TH actions [35,36]. Because of this presumption and keeping in mind the unavailability of scientific literature on the role of PQQ in the regulation of thyroid dysfunctions, the present study was undertaken to evaluate the role of PQQ in ameliorating 6-n-propyl-2-thiouracil (PTU) induced oxidative damage in kidney.

Therefore, the present investigation has been designed to evaluate the effect of PQQ on PTU/ hypothyroidism-induced oxidative damage and lipid peroxidation in kidney of mice. Kidney samples analyzed in the present study were of our experimental model reported earlier [37].

MATERIALS AND METHODS

ANIMALS

Swiss albino mice, weighing 30±2 gm were housed in polypropylene cages in a standard photoperiod (14 h light: 10 h dark) and temperature (27±1°C) controlled room with the provision of laboratory feed (Gold Mohur feed, Hindustan Lever Limited, Mumbai, India) and water *ad libitum*. Animals were maintained in accordance with the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Social justice and Empowerment, Govt. of India. The experimental protocol was reviewed and approved by Institutional Animal Ethics Committee. (Ref. no. 779)

CHEMICALS

PQQ was purchased from Quality of life lab, USA; PTU was obtained from Sigma-Aldrich chemicals (St. Louis, MO, USA). Ellman's reagent, m-phosphoric acid, thio-barbituric acid (TBA), sodium dodecyl sulphate, tri carboxylic acid (TCA), hydrogen peroxide (H_2O_2) were obtained from E. Merck Ltd., Mumbai, India. Kits for the estimation of different lipids, glucose, urea, creatinine and glucose were procured from Transasia Bio-Medicals ltd., Solan, India. All other chemicals were of reagent grade and obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

EXPERIMENTAL DESIGN

Five groups of 7 each healthy male mice were established. Group I animals receiving simple drinking water served as control, whereas those of group II, III, IV and V received only PTU (0.05% in drinking water for 5 weeks) [28,31,34]. On 30th day, animals of group III, IV and V received different doses of PQQ (1, 5 and 10 mg/ kg/ day for six days, respectively) along with PTU as administered to group II animals. Experiment was continued for 5 consecutive weeks. On the day of termination (36th day), over night fasted animals were sacrificed under mild anesthesia, blood from each animal was collected and serum was separated for the estimation of different biochemical parameters including serum concentrations of glutamate oxaloacetate transaminase (SGOT), glucose, creatinine, urea and total cholesterol. After exsanguinations, kidney tissues were removed quickly, washed with phosphate buffered saline (PBS) and processed for the estimation of lipid peroxidation (LPO), super-oxide dismutase (SOD), catalase (CAT) activities and glutathione (GSH) content.

BIOCHEMICAL ESTIMATIONS

Kidney tissues were homogenized in PBS (0.1M, pH 7.4), centrifuged at 15,000g for 30 min at 4^{o}C and the supernatant was used for subsequent analysis.

LIPID PEROXIDATION (LPO)

Lipid peroxidation level in the tissues was measured by the method of Ohkawa et al., 1979 [38] which is based on the TBA reaction with MDA, a product formed due to the peroxidation of membrane lipids. The amount of MDA was measured by taking the absorbance at 532 nm (extinction coefficient, E =1.56x10^5), using a Shimadzu UV-170 spectrophotometer. LPO was finally expressed as nM MDA formed/ h/ mg protein.

SUPER-OXIDE DISMUTASE (SOD) ASSAY

Activity of SOD was determined following the pyrogallol auto-oxidation inhibition assay method of Marklund & Marklund, 1974 [39]. The rate of auto-oxidation is calculated from the increase in absorbance at 420 nm. The enzyme activity was expressed as units /mg protein and 1 unit is defined as the enzyme activity that inhibits auto-oxidation of pyrogallol by 50 %.

CATALASE (CAT) ASSAY

Catalase activity was estimated following the method of Aebi, 1983 [40] that is based on the decomposition of H_2O_2 which is measured spectrophotometrically from the changes in absorbance at 240 nm which was expressed as μM of H_2O_2 decomposed min^{-1} mg^{-1} protein.

GLUTATHIONE (GSH) ASSAY

For the estimation of tissue GSH content the protocol of Ellman, 1959 [41] was followed in which the –SH group of GSH reacts with DTNB to produce a yellow-colored 2-nitro-5-mercaptobenzoic acid and the absorbance was taken at 412 nm. The GSH content is expressed as μM GSH/ mg protein.

PROTEIN, GLUCOSE, TOTAL CHOLESTEROL, UREA AND CREATININE ESTIMATIONS

Protein estimation was done by the routine method of Lowry et al., 1951 [42] using bovine serum albumin as standard and fasting serum glucose concentration was measured by glucose oxidase/ peroxidase method based on the protocol of Trinder, 1969 [43] where 4-amino antipyrine and phenol reacts with glucose to produce a pink colored quinoneimine dye. The intensity of the color developed is proportional to glucose concentration in the sample, while estimations of serum total cholesterol were done using spectrometric methods of Allain et al., 1974 [44]. Urea and creatinine were estimated using the commercially available kits and protocols of Transasia bio-medicals ltd. Solan, India.

SGOT ASSAY

The method used here is of Reitman and Frankel, 1957 [45] colorimetric end point reaction method. SGOT **c**atalyzes transfer of amino group from L-aspartate to α-ketoglutarate with formation of oxaloacetate and glutamate. The oxaloacetate, so formed, is allowed to react with 2,4-DNPH (2,4 dinitro phenyl hydrazine) to form 2,4 dinitro phenyl hydrazone derivative, which is brown colored in alkaline medium. The absorbance of this hydrazone derivative is correlated to SGOT activity by plotting a calibration curve using pyruvate standard. The colored complex is read at 505 nm.

STATISTICAL ANALYSIS

Data are expressed as means ± SEM. For the statistical evaluation, analysis of variance and Student t test were used Senedector and Cochran, 1956 [46]. A p value of 0.05 or less is considered as the level of significance.

RESULTS

PTU administration increased the LPO (Fig. 1) in kidney tissues significantly ($p<0.01$); with a parallel decrease in SOD and CAT ($p<0.05$ respectively). However, there was no significant change in GSH level after PTU administration in kidney tissues.

Following the administration of PQQ interestingly all these effects of PTU were reversed. However, out of the three doses (1, 5 and 10 mg/ kg/ d) of PQQ, 10 mg/kg body weight was found to be most effective in decreasing LPO significantly ($p<0.001$) and the percentage decreases were found to be 60%. Of course other two doses were also found to be significantly effective (Fig. 1).

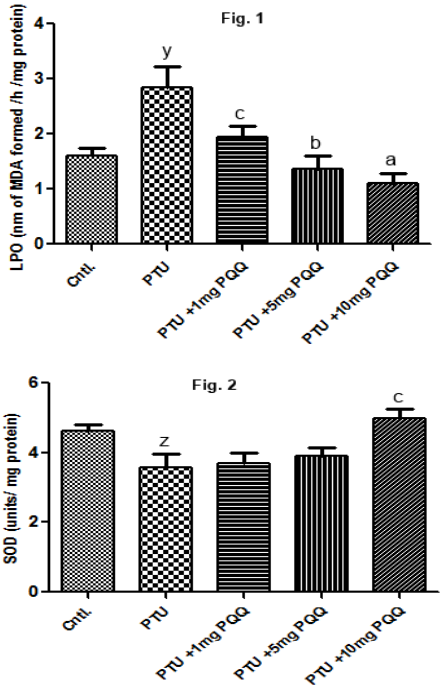
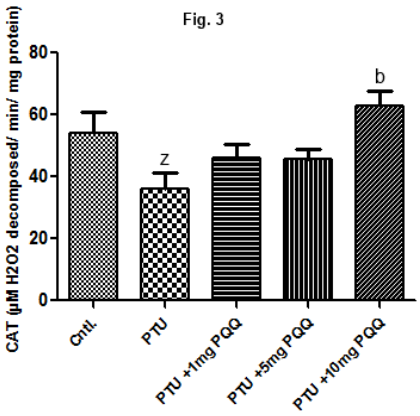


Figure 1: Effects of PQQ (1, 5 and 10 mg/kg/d, i.p.) for 6 days on LPO (nm of MDA formed/ h/ mg protein), and **Figure 2:** Effects of PQQ on SOD (units/ mg protein). Data are mean ± S.E.M. (n=7). ^{y,} $p<0.01$, ^{z,} $p<0.05$ compared to the respective control values. ^{a,} $p<0.001$, ^{b,} $p<0.01$ and ^{c,} $p<0.05$ as compared to the respective value PTU treated group values. ^{PTU,} Propylthiouracil, ^{LPO}, Lipid Peroxidation, ^{SOD,} Superoxide Dismutase and ^{PQQ,} Pyrroloquinoline quinone

With respect to SOD activity, the significant and best effect was noticed only with the highest dose, i.e. 10 mg/kg ($p<0.05$) (Fig. 2). Similar activity was also found with catalase enzyme activity that also significantly increased in the studied tissues ($p<0.01$) by the administration of 10 mg/ kg body weight of PQQ (Fig. 3) and the percentage increases were found to be 75%. Although PTU did not exhibit any significant changes in GSH content, interestingly PQQ increased it significantly ($p<0.001$) at a dose of 10 mg/kg with a percent increase of 158%, while other two doses were also found to enhance it (Fig. 4).



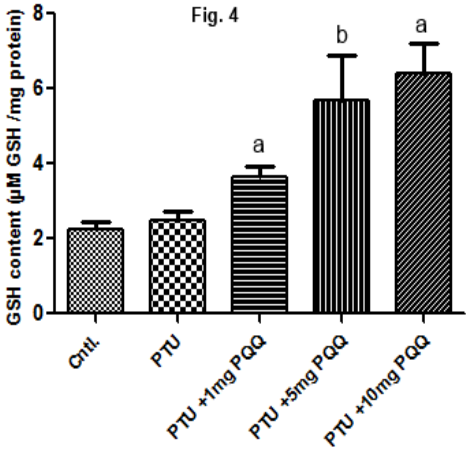


Figure 3: Effects of PQQ (1, 5 and 10 mg/kg/d, i.p.) for 6 days on CAT (µM H_2O_2 decomposed/ min/ mg protein) and **Figure 4:** Effect of PQQ on GSH content (µM GSH/ mg protein). Data are mean ± S.E.M. (n=7). [z,] $p<0.05$ compared to the respective control values. [a,] $p<0.001$ and [b,] $p<0.01$ as compared to the respective value PTU treated group values. [PTU,] Propylthiouracil, [CAT,] Catalase, [GSH,] Reduced glutathione and [PQQ,] Pyrroloquinoline quinone.

With respect to serum parameters, a significant increase in the level of total cholesterol, SGOT, creatinine and urea was found in PTU treated animals ($p<0.01$, $p<0.001$, $p<0.01$ and $p<0.001$ respectively, Fig. 5) However, administration of PQQ at a dose of 10 mg/kg markedly reduced all these indices and the percentage decreases were found to be 33%, 74%, 59% and 56% in total cholesterol, SGOT, creatinine and urea respectively. Following the administration of PTU, level of serum glucose decreased significantly ($p<0.05$ by 25%); while by the simultaneous administration of 5mg and 10 mg/kg of PQQ it increased significantly ($p<0.001$ for both, 26 & 32% respectively). Other two doses were also found to be significantly effective (Table 6).

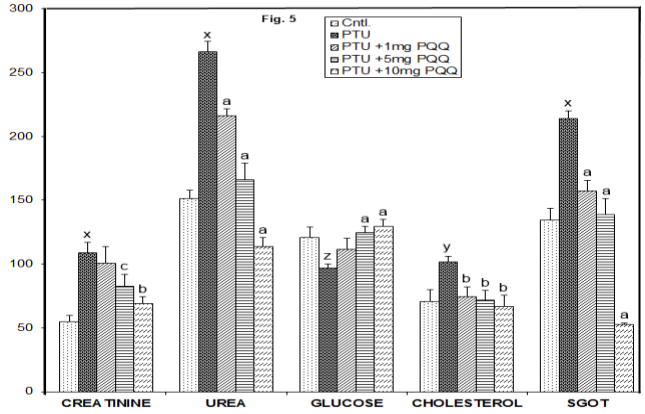


Figure 5: Effects of PQQ (1, 5 and 10 mg/kg/d, i.p.) for 6 days on different serum parameters of creatinine (mg/dl), urea (mg/dl), glucose (mg/dl), total cholesterol (mg/dL) and SGOT (IU/L). Data are mean ± S.E.M. (n=7). [x,] $p<0.001$ [y,] $p<0.01$ and [z,] $p<0.05$ compared to the respective control values. [a,] $p<0.001$, [b,] $p<0.01$ and [c,] $p<0.05$ as compared to the respective value PTU treated group values. [PTU,] Propylthiouracil, [PQQ,] Pyrroloquinoline quinone, [SGOT,] Serum glutamate oxaloacetate transaminase.

DISCUSSION

From the results of the present experiment it is clearly revealed that, PQQ has the potential in regulating the PTU induced oxidative damage in kidney of mice and other associated problems including serum glucose, cholesterol, creatinin, urea and SGOT. Administration of PTU, a commonly used antithyroidic drug significantly increased lipid peroxidation in kidney with a parallel decrease in the antioxidants such as SOD and CAT in the similar manner as reported earlier by others [30,34]. However, a PTU-induced animal when received PQQ simultaneously reversed these effects, suggesting that it has the potential to ameliorate PTU/ hypothyroid- induced adverse effects.

Thyroid hormones are among the most important factors involved almost all functional aspects of the body, including metabolic, respiratory, cardiovascular, nervous and reproductive functions, either directly or indirectly and PTU inhibits thyroid functions [28,29]. *In vivo* cellular oxidative stress is also associated with thyroid abnormalities. It is further known that deficiency of thyroid hormones can lead to an oxidative stress condition in the kidney with a consequent lipid peroxidative response [47]. Moreover, hypothyroidism-induced dysfunction of the respiratory chain in the mitochondria leads to accelerated production of free radicals (i.e., superoxide anion, hydrogen peroxide, and hydroxyl radical as well as lipid peroxides), which consequently leads to oxidative stress [47-49]. Interestingly, in the present study, following the administration of PQQ, particularly 10 mg/ kg, not only LPO was reduced in kidney, but also levels of cellular antioxidants such as SOD, CAT and GSH were enhanced suggesting the antioxidative potential of the PQQ. These observations corroborate with the earlier reports, where free radical scavenging property of PQQ has been highlighted [12,50].

Some workers suggest that hypothyroidism protects tissues against accelerated lipid peroxidation, although the data concerning oxidation / antioxidation in hypothyroidism are incomplete and contradictory [30,51]. In fact, Dariyerli et al., 2004 [52] did not find any change in the content of malondialdehyde in rats with thiamizole-induced hypothyroidism. Hypothyroidism specifically reduces most tissues cellular thiol reserves and alters glutathione content. GSH is a well-known antioxidant that provides the major protection against cellular oxidative damages and maintains SH level in proteins [53]. In this experiment, GSH level remained unchanged in PTU administered group in comparison to control as observed earlier by some other workers [54,55]. However, administration of PQQ increased the GSH level, suggesting that the test compound has the potential to enhance cellular antioxidant status.

PQQ is highly electrophilic in nature and it reacts with many substances. It forms stable adducts with carbonyl reagents. These characteristics provide PQQ the ability to oxidize the redox modulatory site, thus conferring protection against ROS-mediated cell injury [23]. The possibility of PQQ-induced reduction in intracellular ROS levels is consistent with the hypothesis that it acts directly or indirectly as a potent free radical scavenger. Probably for this reason LPO level was normalized in PQQ treated animals. Of course its site of action, either intra-mitochondrial or in the cytoplasm or both, is yet to be determined [56]. This could be the mode of action of PQQ in the present study also. The positive effects of PQQ were also reflected in the PTU induced animals with respect to other indices such as serum glucose, total cholesterol, creatinine and urea; particularly at a concentration of 10 mg/ kg. When percentage of increase and decrease in different indices were calculated, better effects were also observed at 10 mg/ kg as compared to the other two concentrations.

An increase of lipid peroxidation attributed to dyslipidemia has also been observed in women with hypothyroidism [57]. Both qualitative and quantitative lipid disorders, hyperlipoproteinemia and hypercholesterolemia have been described for a long time in hypothyroidism [58]. Since we also observed hypercholesterolemia with a parallel increase in tissue LPO, the observed PTU induced increase in tissue LPO may be considered as the result of hypothyroid condition.

In our study the elevation of both serum urea and creatinine levels in response to PTU indicates possible renal damage. These two are considered as the major indices of impaired kidney functions [59] and their increased level in the serum of hypothyroid rats may be due to reduction in glomerular filtration rate (GFR) [60]. Interestingly administration of PQQ to PTU treated mice resulted in declined level of serum urea and creatinine as compared to PTU treated animals, again suggesting the protective effects of the test compound. The beneficial effect of PQQ was also reflected in the alterations in the activities of SGOT. While in PTU administered animals there was an increase in this enzyme activity, it was reversed by simultaneous administration of PQQ.

Kidney is a major target organ for thyroid hormone with important biological and medical implications [61,62]. Clinical diagnosis of disease and damage to the structural integrity of kidney is commonly assessed by monitoring the status of serum SGOT activities [63]. In fact, enzymatic activities of SGOT are considered as sensitive serological indicators of kidney, muscle and brain toxicity. In our study these parameters were significantly altered by PTU, suggesting that PTU might cause critical injury to kidney and other organs. These observations along with change in LPO indicated that hypothyroidism may lead to the overproduction of free radicals, which in turn exert deleterious effects on kidney and other organs. As following the administration of PQQ in PTU administered mice, serum SGOT levels were reduced to near normal values, the positive effects of the test drug was again supported.

With respect to the alterations in different lipid contents, PTU administration increased the total cholesterol concentrations in PTU treated animals. Earlier Plisetskaya et al., 1983 [64] have shown that chemical thyroidectomy by the treatment of thiourea or 6-PTU causes either depletion or accumulation of liver lipids. It is also understood that the variations in the serum total cholesterol is very often related to thyroid dysfunctions altering cholesterol biosynthesis. The most noticeable fact is that hypothyroidism results in the accumulation of cholesterol in the body [65,66]. Hyperlipedemia and hypercholesterolemia may result from increased mobilization of body fat reserves due to increased thyrotrophic hormone level and hypothyroidism [67,68]. Low thyroxin level in hypothyroid animals not only triggers enhanced thyrotropin secretion from pituitary but also stimulates corticotrophin, and in turn, adrenal steroids, thereby increases lipid mobilization through overlapping endocrine axis [69]. In fact, hypercholesteremia, hyperlipidemia and increase in body weight have been suggested as excellent indicators of decreased thyroid function [70]. Interestingly, in our study all these PTU induced adverse effects including increase in body weight were ameliorated by the simultaneous administration of PQQ, clearly suggesting the potential of test drug in ameliorating hypothyroidism.

This may be emphasized that nothing was known on the role of PQQ in regulating hypothyroidism. Therefore, our study can be compared with the other antioxidative plant based active compounds, which were also found to be beneficial in the regulation of hypothyroidism in animal model [71,72]. In fact, till to date no literature is available in relation to the role of PQQ in the regulation of PTU-induced renal dysfunction. Therefore, the present report appears to be the first one that clearly indicates the efficacy of PQQ in regulating PTU induced abnormalities in kidney, despite the fact that PQQ was earlier known to regulate different abnormalities including cardiac problems [73] which are often related with thyroid dysfunctions. Now our findings ascertain that PQQ has the potential to regulate PTU induced adverse effects/ hypothyroidism.

The possible mechanism of its efficacy to ameliorate PTU induced oxidative stress could be its strong antioxidant properties, as suggested in some previous studies, in which the free radical scavenging activity of PQQ has been clearly shown [11,26]. As decrease in tissue lipid peroxidation and increase in superoxide dismutase and catalase activities coincided with elevation in thyroid hormones, it is quite possible that the toxic/ adverse effect of PTU might have been ameliorated by PQQ through the alterations in thyroid hormones, because thyroid hormones are known to reduce tissue lipid peroxidation and increase the levels of natural antioxidants [74]. Whatever may be the mechanism of action, from our present findings it is clearly evident that PQQ has the potential to ameliorate PTU induced oxidative stress in kidney and it may work against hypothyroidism. Further investigation is required for better understanding on this aspect.

CONCLUSION

Findings from this study revealed for the first time, that PQQ has the potential to ameliorate PTU-induced oxidative damage in kidney of mice, indicating the possible beneficial effect of the test compound in regulating PTU induced oxidative damages. Of course out of three doses of PQQ (1, 5 and 10 mg/kg/d), 10 mg/kg body weight was found to be the most effective.

ACKNOWLEDGEMENT

Financial support from the Council of Scientific and Industrial Research (CSIR), New Delhi, India for a Senior Research fellowship to Narendra Kumar is gratefully acknowledged.

CONFLICT OF INTEREST

The authors declare no conflict of interest

REFERENCES

1. Duine JA, Frank J: Quinoprotein alcohol dehydrogenase from a non-methylotroph, Acinetobacter calcoaceticus. J Gen Microbiol. 1981;122:201–209.
2. Salisbury SA, Forrest HS, Cruse WB, Kennard O: A novel coenzyme from bacterial primary alcohol dehydrogenases. Nature. 1979;280:843–844.
3. Ameyama M, Matsushita K, Ohno Y, Shinagawa E, Adachi O: Existence of a novel prosthetic group PQQ, in membrane-bound, electron transport chain-linked, primary dehydrogenases of oxidative bacteria. FEBS Lett. 1981;130:179–183.
4. Van Kleef MAG, Duine JA: L-Tyrosine is the precursor of PQQ biosynthesis in Hypomicrobium X. FEBS Lett. 1988;237:91-97.
5. Kobayashi K, Mustafa G, Tagawa S, Yamada M: Transient formation of a neutral ubisemiquinone radical and subsequent intramolecular electron transfer to pyrroloquinoline quinone in the *Escherichia coli* membrane-integrated glucose dehydrogenase. Biochemistry. 2005;44:13567–13572.
6. Khairnar NP, Kamble VA, Mangoli SH, Apte SK, Misra HS: Involvement of a periplasmic protein kinase in DNA strand break repair and homologous recombination in *Escherichia coli*. Mol Microbiol. 2007;65:294–304.

7. Choi O, Kim J, Kim JG, Jeong Y, Moon JS, Park CS, et al. Pyrroloquinoline quinone is a plant growth promotion factor produced by Pseudomonas fluorescens B16. Plant Physiol. 2008;146:657–668.
8. Yamaguchi K, Sasano A, Urakami T, Tsuji T, Kondo K: Stimulation of nerve growth factor production by pyrroloquinoline quinine and its derivatives *in vitro* and *in vivo*. Biosci Biotechnol Biochem. 1993;57:1231–1233.
9. Andrea CR, Antonio R, Augusto R, Enrico S: Modeling novel quinocofactors: An overview. Bioorg Chem. 1999;27:253–288.
10. Rucker R, Chowanadisai W, Nakano M: Potential Physiological Importance of Pyrroloquinoline Quinone. Altern. Med Rev. 2009;14:268-277.
11. Misra HS, Rajpurohit YS, Khairnar NP: Pyrroloquinoline-quinone and its versatile roles in biological processes. J Biosci. 2012;37:313–325.
12. Misra HS, Khairnar NP, Barik A, Indira Priyadarsini K, Mohan H, Apte SK: Pyrroloquinoline-quinone: a reactive oxygen species scavenger in bacteria. FEBS Lett. 2004;578:26–30.
13. Rajpurohit YS, Gopalakrishnan R, Misra HS: Involvement of a protein kinase activity inducer in DNA double strand break repair and radioresistance of Deinococcus radiodurans. J Bacteriol. 2008;190:3948–3954.
14. Zhu B, Zhou H, Teerlink JR, Karliner JS: Pyrroloquinoline Quinone (PQQ) Decreases Myocardial Infarct Size and Improves Cardiac Function in Rat Models of Ischemia and Ischemia/Reperfusion. Cardiovasc Drugs Ther. 2004;18:421–431.
15. Wu QS, Wang JH, Zhou YH, Qin F, Shen L, Wu SL: Effect of pyrroloquinoline quinoneon free radical in rats with radiation induced skin injury. J Jiangsu Univ (Med. Ed.). 2008;18:403–405.
16. Qiu XQ, Liu CL, Xu L, Zhao JY, Wu SL: Effect of pyrroloquinoline quinone on antioxidative competence of AGS cell radiated by γ-ray. J Jiangsu Univ (Med. Ed.) 2009;19:293–295.
17. Gallop PM, Paz MA, Flückiger R, Henson E: Is the antioxidant, anti-inflammatory putative new vitamin, PQQ, involved with nitric oxide in bone metabolism? Connect Tissue Res. 1993;29:153-161.
18. Hara H, Hiramatsu H, Adachi T: Pyrroloquinoline quinine is a potent neuroprotective nutrient against 6-hydroxydopamineinduced neurotoxicity. Neurochem Res. 2007;32:489–495.
19. Nunome K, Miyazaki S, Nakano M, Iguchi-Ariga S, Ariga H: Pyrroloquinoline quinine prevents oxidative stress-induced neuronal death probably through changes in oxidative status of DJ-1. Biol Pharm Bull. 2008;31:1321–1326.
20. Zhang P, Xu Y, Li L, Jiang Q, Wang M, Jin L: In vitro protective effects of pyrroloquinoline quinone on methylmercury-induced neurotoxicity. Environ Toxicol Pharmacol. 2009;27:103–110.
21. Hamagishi Y, Murata S, Kamei H, Oki T, Adachi O, Ameyama M: New biological properties of PQQ and its related compounds: inhibition of chemiluminescence, lipid peroxidation and rat paw edema. J Pharmacol Exp Ther. 1990;255:980-985.
22. Smidt CR, Steinberg FM, Rucker RB: Physiologic importance of pyrroloquinoline quinine. Proc Soc Exp Biol Med. 1991;197:19–26.
23. Aizenman E, Hartnett KA, Zhong C, Gallop PM, Rosenberg PA: Interaction of the putative essential nutrient pyrroloquinoline quinone with the N-methyl-D-aspartate receptor redox site. J Neurosci. 1992;12:2362-2369.
24. Bishop A, Gallop PM, Karnovsky ML: Pyrroloquinoline quinone: a novel vitamin? Nutr Rev. 1998;56:287–293.
25. Jensen FE, Gardner GJ, Williams AP, Gallop PM, Aizenman E, Rosenberg PA: The putative essential nutrient pyrroloquinoline quinone is neuroprotective in a rodent model of hypoxic/ischemic brain injury. Neurosci. 1994;62:399–406.
26. Ouchi A, Nakano M, Nagaoka S, Mukai K: Kinetic study of the antioxidant activity of pyrroloquinolinequinol (PQQH(2), a reduced form of pyrroloquinolinequinone) in micellar solution. J Agric Food Chem. 2009;57:450-456.
27. Xiong XH, Zhi JJ, Yang L, Wang JH, Zhao Y, Wang X, et al. Complete genome sequence of the bacterium Methylovorus sp. strain MP688, a high-level producer of pyrroloquinolone quinone. J Bacteriol. 2011;193:1012–1013.
28. Guerrero A, Pamplona R, Postero-Otin M, Barja G, Lopez-Torres M: Effect of thyroid status on lipid composition and peroxidation in the mouse liver. Free Rad Biol Med. 1999;26:73-80.
29. Ganong WF: Review of Medical Physiology. 2005; 22nd edn. Appleton & Lange, Connecticut.
30. Venditti P, Di Meo S: Thyroid hormone-induced oxidative stress. Cell Mol Life Sci. 2006;63:414–434.
31. Messarah M, Boumendjel A, Chouabia A, Klibet F, Abdennour C, Boulakoud MS, et al. Influence of thyroid dysfunction on liver lipid peroxidation and antioxidant status in experimental rats. Exp Toxicol Pathol. 2010;62:301–310.
32. Asayama K, Kato K: Oxidative muscular injury and its relevance to hyperthyroidism. Free Radic Biol Med. 1990;8:293–303.
33. Das K, Chainy GB: Modulation of rat liver mitochondrial antioxidant defence system by thyroid hormone. Biochim Biophys Acta. 2001;1537:1–13.
34. Jena S, Dandapat J, Chainy GB: Curcumin differentially regulates the expression of superoxide dismutase in cerebral cortex and cerebellum of L: thyroxine (T4) induced hyperthyroid rat brain Neurol Sci. 2012;34:505-510.
35. Kaptein EM, Feinstein EI, Massry SG: Thyroid hormone metabolism in renal diseases. Contribut Nephrol. 1982;33:122–135.
36. den Hollander JG, Wulkan RW, Mantel MJ, Berghout A: Correlation between severity of thyroid dysfunction and renal function. Clin Endocrinol. 2005;62:423–427.
37. Subudhi U, Das K, Paital B, Bhanja S, Chainy GBN: Supplementation of curcumin and vitamin E enhances oxidative stress, but restores hepatic histoarchitecture in hypothyroid rats. Life Sci. 2009;84:372–379.
38. Ohkawa H, Ohishi N, Yagi K: Assay of lipid peroxides in animal tissues by thiobarbiturie acid reaction. Anal Biochem 1979;95:351-358.
39. Marklund S, Marklund G: Involvement of superoxide anion radical in the autoxidation of pyrogallol: A convenient assay for superoxide dismutase. Eur J Biochem. 1974;47:469-474.
40. Aebi HE: Catalase. In: Bergmeyer HU (ed.). Methods in Enzymatic Analysis. Academic Press, New York. 1983; 276–286.
41. Ellman GL: Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82:70–77.
42. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ: Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265-275.
43. Trinder P: Determination of blood glucose using an oxidase–peroxidase system with a non-carcinogenic chromogen. J of Clin Pathol. 1969;22:158–161.
44. Allain CC, Poon LS, Chan CS, Richmond W, fu PC: Enzymatic determination of total serum cholesterol. Clin Chem. 1974;20:470-475.
45. Reitmen S, Frankel S: Acolorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am J Clin Pathol. 1957;28:56–63.
46. Snedecor GW, Cochran WG: Statistical methods applied to experiments in agriculture and biology. 5th ed. Ames, Iowa: Iowa State University Press, 1956, pp 534.
47. Yilmaz S, Ozan S, Benzer F, Canatan H: Oxidative damage and antioxidant enzyme activities in experimental hypothyroidism. Cell Biochem Funct. 2003;21:325–330.
48. Venditti P, Balestrieri M, Di Meo S: Effect of thyroid state on lipid peroxidation, antioxidant defences, and susceptibility to oxidative stress in rat tissues. J Endocrinol. 1997;155:151–157.
49. Carmeli E, Bachar A, Barchad S, Morad M, Merrick J: Antioxidant status in the serum of personswith intellectual

disability and hypothyroidism: Apilot study. Res. Development. Disab. 2008;29:431-438.
50. Shrivastava M, Rajpurohit YS, Misra HS, D'Souza SF: Survival of phosphate-solubilizing bacteria against DNA damaging agents. Can J Microbiol. 2010;56:822–830.
51. Isman CA, Yegen BC, Alican I: Methimazole-induced hypothyroidism in rats ameliorates oxidative injury in experimental colitis. J Endocrinol. 2003;177:471–476.
52. Dariyerli N, Toplan S, Akyolcu MC, Hatemi H, Yigit G: Erythrocyte osmotic fragility and oxidative stress in experimental hypothyroidism. Endocrine. 2004;25:1–5.
53. Sies H; Glutathione and its role in cellular functions. Free Radic Biol Med. 1999;27:916–921.
54. Asayama K, Dobashi K, Hayashibe H, Megata Y, Kato K; Lipid peroxidation and free radical scavengers in thyroid dysfunction in the rat: a possible mechanism of injury to heart and skeletal muscle in hyperthyroidism. Endocrinology. 1987;121:2112–2118.
55. Tas S, Dirican M, Sarandol E, Serdar Z: The effect of taurine supplementation on oxidative stress in experimental hypothyroidism. Cell Biochem Funct. 2006;24:153–158.
56. Tao R, Karliner JS, Simonis U, Zheng J, Zhang J, Honbo N, Alano CC: Pyrroloquinoline quinone preserves mitochondrial function and prevents oxidative injury in adult rat cardiac myocytes. Biochem Biophys Res Commun. 2007;363:257-262.
57. Nanda N, Bobby Z, Hamide A, Koner BC, Sridhar MG: Association between oxidative stress and coronary lipid risk factors in hypothyroid women is independent of body mass index. Metabolism. 2007;56:1350–1355.
58. Wahlqvist ML, Fidge NH, Lomas F: Lipoprotein composition in hypothyroidism. Clin Chim Acta. 1977;77:269–274.
59. Lindeman RD: Overview: renal physiology and pathology of aging. Am J Kidney Dis. 1990;4:275–282.
60. Montenegro J, Gonzalez O, Saracho R, Aguirre R, Gonzalez O, Martinez I: Changes in renal function in primary hypothyroidism. Am J Kidney Dis. 1996;27:195–198.
61. Feng X, Jiang Y, Melzer P, Yen P M: Thyroid hormone regulation of hepatic genes in vivo detected by complementary DNA microarray. Mol Endocrinol. 2000;14:947–955.
62. Simon-Giavarotti KA, Giavarotti L, Gomes LF, Lima AF, Veridiano AM, Garcia EA, et al. Enhancement of lindane-induced liver oxidative stress and hepatotoxicity by thyroid hormone is reduced by gadolinium chloride. Free Radic Res. 2002;36:1033–1039.
63. Amin A, Hamza AA: Oxidative stress mediates drug-induced hepatotoxicity in rats: a possible role of DNA fragmentation. Toxicology. 2005;208:367–375.
64. Plisetskaya E, Woo NYS, Murat J: Thyroid hormones in cyclostomes and fish and their role in regulation of intermediary metabolism. Comp Biochem Physiol A Mol Integr Physiol. 1983;74:179–187.
65. Norris DO: Depression of growth following radiothyroidectomy of larval Chinook salmon and steelhead trout. Trans Am Fish Soc. 1969;98:104–106.
66. Sheridan MA, His Kao Y: Regulation of metamorphosis associated changes in the lipid metabolism of selected vertebrates. Am Zool. 1998;38:350–368.
67. Varghese S, Oommen OV: Thyroid hormones regulate lipid metabolism in teleost Anabas testudinens (Bloch). Comp Biochem Physiol. 1999;124:445–450.
68. Varghese S, Shameena B, Oommen OV: Thyroid hormones regulate lipid peroxidation and antioxidant enzyme activities in Anabas testudinens (Bloch). Comp Biochem Physiol. 2001;128:165–171.
69. Mc Donald LE: Veterinary endocrinology and reproduction, 3rd ed. London: Lea and Febiger, Philadephia/Balliere Jindall Publication;1980;42–59.
70. Al-Tonsi AA, Abdel-Gayoum AA, Saad M: The secondary dyslipidemia and deranged serum phosphate concentration in thyroid disorders. Exp Mol Pathol. 2004;76:182–187.
71. Panda S, Kar A: Guggulu (*Commiphora mukul*) potentially Ameliorates Hypothyroidism in female mice. Phytoth Res. 2005;19:78-80.
72. Jatwa R, Kar A: Amelioration of metformin-induced hypothyroidism by *Withania somnifera* and *Bauhinia purpurea* extracts in type 2 diabetic mice. Phytoth Res 2009;23:1140-1145.
73. Zhu B, Zhou H, Teerlink JR, Karliner JS: Pyrroloquinoline Quinone (PQQ) Decreases Myocardial Infarct Size and Improves Cardiac Function in Rat Models of Ischemia and Ischemia/Reperfusion. Cardiovasc Drugs Ther. 2004;18:421–431.
74. Maiti PK, Kar A: Is triiodothyronine capable of ameliorating pyrethroid-induced thyroid dysfunction and lipid peroxidation. J Appl Toxicol. 1998;18:125–128.