IJPSR (2022), Volume 13, Issue 4 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 26 July 2021; received in revised form, 15 November 2021; accepted, 23 March 2022; published 01 April 2022

GLUTATHIONE AND ANTIOXIDANT STUDY IN TESTES OF WISTAR RATS AFTER DIETARY COPPER DEFICIENCY

Aastha Saini, Ankita Rajendra Kurup and Neena Nair *

Department of Zoology, Centre for Advanced Studies, University of Rajasthan - 302004, Jaipur, India.

Keywords:

Copper deficiency, Testes, glutathione, Total SOD, Cu-Zn SOD, Mn SOD, Catalase

Correspondence to Author:
Prof. Neena Nair

Professor,
Department of Zoology, Centre for Advanced Studies, University of Rajasthan - 302004, Jaipur, India.

E-mail: neenazoology@yahoo.co.in

ABSTRACT: Copper is an essential trace element involved in a plethora of functions being a component of several enzymes. Copper deficiency affects people of all age groups, including infants, children, pregnant women, and men. Deficiency occurs due to numerous factors hindering the absorption/uptake of copper. Male Wistar rats (35-50 gm) were divided into four groups: Group 1: Negative control – fed with standard feed (Ashirwad Industries, Chandigarh) and tap water was provided ad *libitum* .Group 2: Copper control group- Fed with diet containing 126 nmol Cu/gm. Tap water was provided *ad libitum*. (c) Group 3: Pair-fed group - Fed with 126 nmol Cu /gm diet but the amount of feed given was equal to the feed consumed by the copper-deficient group the previous day to account for stress and starvation effect caused due to low intake diet. Tap water was provided *ad libitum* (d) Group 4: Copper deficient- animals were fed with 6.3 nmol Cu / gm diet, and demineralized water was provided *ad libitum*. The experiment was conducted for 2-, 4- and 6 weeks. Glutathione concentration and Cu-Zn SOD decreased significantly ($P<0.05$) after dietary copper deficiency. After copper deficiency, a significant increase ($P<0.05$) in total SOD, Mn-SOD, and catalase activities /expression was observed. The vulnerability of testes to oxidative stress and subsequent generation of reactive oxygen species accounts for impaired glutathione concentration and antioxidant enzymes. This indicates that prolonged perturbation of the copper homeostasis would hamper the functional aspect of testes ultimately leading to infertility.

INTRODUCTION: Micronutrient deficiency is a global problem, especially where the intake of food is cereals which are already low in micronutrients. The problem is further enhanced with these cereals grown in deficient soil, which further reduces its concentration. Copper deficiency is on the rise because soils do not respond to copper added in fertilizers and range to 10- 15% in India [1]. Fodders have been reported to have low copper content, which would impact animals and ultimately human beings [2, 3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(4).1747-54

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(4).1747-54

Kohlaa *et al.*,[4] reported breastfeeding a full-term female infant with severe copper deficiency. Deficiency of copper can also occur due to antagonistic action of molybdenum, sulfur, iron, excess zinc, malabsorption, foregut surgery, and hereditary disorders [5, 6, 7].

Several authors [8, 9] reported average daily intakes of copper in the range of 0.8 - 3 mg, with the absorbed copper being bound to albumin, histidine/glutathione. Copper has the ability to react with superoxide anion and hydrogen peroxide leading to the formation of hydroxyl radical [10, 11]. Oxidative stress generation of excess reactive oxygen species (ROS) is one of the major causes of male infertility. Enhanced ROS has profound effects on cells, Gene expression and genetic integrity of spermatozoa [12, 13] with an ability to

induce lipid peroxidation, damage DNA, RNA, and functions of testicular cells [14]. In testes, Sertoli cells and spermatozoa have been reported to generate reactive oxygen species [15, 16]. Oxidative damage in testes has been reported to occur due to prolonged exposure to stress [17, 18]. To eliminate these toxic effects mammals / aerobic organisms have efficient mettaloenzymes glutathione peroxidase, superoxide dismutase and catalase [19-22]. Glutathione (GSH), a tripeptide of L- γ –glutamyl –L–cysteinyl glycine abundantly found in mammalian tissues in the range of approximately 0.1-15 mM and has the potential to reduce the level of reactive oxygen species as well as reactive nitrogen species [23-27].

Most of the copper absorbed by the body is bound mainly to metallothionein glutathione [28]. Glutathione - hydrophilic antioxidant functions to (i) neutralize hydrogen peroxide (ii) secondary lipid peroxidation products formed during damage to the tissue [29, 30] and (iii) involved in signaling and also scavenges NO [31, 32, 33]. Copper, an important component of superoxide dismutase (SOD, EC1.15.1.1) considered as the first line of antioxidant defense [34], has a significant role in male reproductive organs protecting the cellular structure and function against oxidative damage. Trist *et al.*,[11] observed that SOD 1 protein provides approximately 90% of total SOD activity in mammalian somatic cells. However, Gu *et al.*,[35] reported 0.73, 0.80 and 0.93 kB SOD1 transcript in mice testes with 0.80 and 0.93 Kb testicular-tSod1 transcripts specific only for the germ cells. Cu/Zn SOD protects against oxidative stress by catalyzing the dismutation of superoxide generated under normal aerobic metabolism. Both transition metals are required for their catalytic activity; although zinc no doubt can be replaced by other metals but copper is critical for the functioning of the enzyme [36].

Intense Cu/Zn SOD protein expression was observed in spermatogonia of human testes, although the expression was weak in other germ cells and somatic cell – Sertoli cells [37]. Manganese (Mn), a component of SOD and psuedocatalase has a role in the redox process [38]. Catalase (EC 1.11.1.6) catalyzes the dismutation of H_2O_2 – harmful reactive species to oxygen and water, although H_2O_2 also functions as a second messenger involved in altered morphology, proliferation, signaling and apoptosis [39]. The metabolization of H_2O_2 occurs both by catalase as well as glutathione peroxidase [10, 40]. The study evaluates the effect of dietary copper deficiency on testes of Wistar rats.

MATERIALS AND METHODS:

Synthetic Diet: The diets were formulated by using ICN Research Diet Protocol (1999). The ingredients of the diet (per kg diet) were- Egg white/ albumin- 180 gm, Corn oil- 100 gm, Corn starch- 443 gm, Sucrose- 200 gm, Cellulose -30 gm, Choline chloride- 2 gm, DL-methionine- 7 gm, AIN- 76 salt mixture- 35 gm, AIN- 76C vitamin-antibiotic mixture- 10 gm. Copper contents of basal diet for each group were estimated at 324.8 nm in air acetylene flame on GBC 902 atomic absorption spectrophotometer, and copper concentrations were adjusted to 126 nmol/gm and 6.3 nmol/gm of copper sulfate.

Experimental Protocol: Male Wistar rats (30-40 days- prepubertal period; 35-50 gm) were divided into four groups with 10 animals in each group: Group 1: Negative control – fed with standard feed (Ashirwad Industries, Chandigarh) and tap water was provided *ad libitum*. Group 2: Copper control / CC group- Fed with diet containing 126 nmol Cu/gm. Tap water was provided *ad libitum*. (c) Group 3: Pair-fed group / PF group- Fed with 126 nmol Cu /gm diet, but the amount of feed given was equal to the feed consumed by the copper-deficient group the previous day to account for stress and starvation effect caused due to low intake diet. Tap water was provided *ad libitum* (d) Group 4: Copper deficient / CD group- animals were fed with 6.3 nmol Cu / gm diet and demineralized water was provided *ad libitum*. Male Wistar rats were housed in isolation in polypropylene cages with stainless steel grills.

Cages and water bottles were washed with detergent solution, demineralized water, and rinsed in 1% EDTA solution prepared in demineralized water to remove copper traces. Experiments were conducted for 2-, 4- and 6-weeks and approved by the University Department Ethics Committee and Committee for Control and Supervision of Experiments on Animals [CPCSEA (1678/GO/Re/S/12)]. After completing the experiments, animals were anesthetized; testes

were excised, cleaned off of extraneous tissue, weighed on Sartorius BS 124 S electronic balance, and processed for biochemical studies.

Biochemical Studies: Testes were homogenized in Remi tissue homogenizer and centrifuged at 2000 rpm (Remi –R8C laboratory centrifuge) or 10,000 rpm (Sigma refrigerated high-speed centrifuge 4 K15 using 18015 rotar) to remove cell debris. Glutathione (GSH) [41, 42]. Superoxide Dismutase (SOD): (A) Total SOD [43], (B) Cu-Zn SOD [44] and (C) Mn-SOD (Total SOD –Cu-Zn SOD) and Catalase [45] were analyzed. The absorbance was read on Systronics spectrophotometer 169 (Serial No. 827).

Statistical Analysis: Data expressed as mean ± SEM. One way Analysis of Variance (ANOVA) was carried out separately for 2-, 4- and 6- week experimental groups and post- hoc test (Tukey's Multiple Comparison test) was carried out if the difference was found to be significant. Data were analyzed using Graph Pad Prism Version 7.0e. $P < 0.05$ was examined to be significant.

RESULT: Glutathione concentration decreased significantly ($P<0.05$) after 2-, 4- and 6- weeks of the experiment when copper deficient (CD) groups were compared with their respective negative control (NC) , copper control (CC), and pair-fed (PF) groups. The decrease was also significant when pair-fed groups of 2- and 6-week experiments were compared with respective NC and CC groups. However, after 4 weeks the decrease was non-significant compared to NC and CC groups **Table 1.**

TABLE 1: BIOCHEMICAL ANALYSIS IN TESTES OF WISTAR RAT AFTER 2-,4- AND 6- WEEKS OF DIETARY COPPER-DEFICIENT DIET (MEAN ± SEM)

Groups	Glutathione (GSH) (µM GSH /mg)	Superoxide Dismutase			Catalase (Kat.f)
		Total SOD (Units mg protein /hr)	Cu-Zn SOD (Units mg protein /hr)	Mn SOD(Units mg protein / hr)	
2NC	14.63±0.061	7.33±0.17	5.45±0.12	1.87±0.28	0.2173±0.0083
2CC	14.62±0.063	7.34±0.18	5.44±0.14	1.90±0.29	0.2206±0.0030
2PF	13.82±0.052 b*d*	8.08±0.14 b*d*	4.32±0.19 b*d*	3.76±0.31 b*d*	0.2839±0.0031 b*d*
2CD	13.55±0.051 c*e*f*	9.09±0.11 c*e*f*	3.55±0.37 c*e*f*	5.54±0.35 c*e*f*	0.3971±0.0083 c*e*f*
4NC	14.90±0.084	10.145±0.12	6.75±0.20	3.38±0.27	0.2494±0.0052
4CC	14.73±0.048	10.146±0.12	6.74±0.26	3.39±0.33	0.2459±0.0031
4PF	14.65±0.050	11.24±0.17 b*d*	3.68±0.14 b*d*	7.54±0.14 b*d*	0.3169±0.0054 b*d*
4CD	12.7±0.175 c*e*f*	15.58±0.30 c*e*f*	2.07±0.13 c*e*f*	13.50±0.27 c*e*f*	0.6339±0.0110 c*e*f*
6NC	18.32±0.040	19.441±0.23	11.38±0.61	8.05±0.58	0.2951± 0.0195
6CC	18.22±0.057	19.443±0.23	11.40±0.49	8.03±0.36	0.2854±0.00696
6PF	17.60±0.054 b*d*	21.43±0.44 b*d*	2.49±0.37 b*d*	18.93±0.37 b*d*	0.3345±0.0065 b*d*
6CD	11.36±0.044 c*e*f*	36.21±0.27 c*e*f*	1.82±0.15 c*e*f*	34.38±0.31 c*e*f*	0.903±0.0029 c*e*f*

a= NC Vs CC, b = NC *vs* PF, * P<0.05 Significantc= NC vs CD, d=CC Vs PF, e =CC Vs CD, f =PF Vs CD Multiple comparison procedures were performed for 2-, 4- and 6 weeks experimental groups.

After 2-, 4- and 6- weeks of dietary copper deficiency total SOD activity increased significantly ($P<0.05$) when copper-deficient groups were compared with their respective NC, CC and PF groups. Comparison of three experimental PF groups with respective NC and CC groups revealed a significant increase ($P<0.05$) **Table 1.** Cu-Zn SOD activity decreased ($P<0.05$) significantly after 2-, 4- and 6- weeks of dietary copper deficiency when copper-deficient groups were compared with their respective NC.CC and PF groups. The decrease was also significant ($P<0.05$) when PF groups(2-, 4- and 6- weeks experiment) were compared with their respective NC and CC groups **Table 1.** Mn-SOD activity increased significantly ($P<0.05$) when copper-deficient groups (2-,4- and 6- weeks experiment) were compared with their respective NC, CC, and PF groups.

A similar pattern of increase was evident when pair-fed groups of the three experiments was compared with their respective NC and CC groups **Table 1.** Catalase activity increased significantly ($P<0.05$) when copper-deficient groups of 2-,4- and 6- weeks experiments were compared with their respective NC, CC and PF groups. A similar pattern of increase was observed when pair-fed

groups of the three experiments was compared with their respective NC and CC groups **Table 1.**

DISCUSSION: Mammalian testes are highly sensitive to oxidative stress with consequent generation of reactive oxygen species due to disruption of cellular redox homeostasis. Non-enzymatic and enzymatic defense systems achieve cellular survival.

Wistar rat testes after a short duration of dietary copper deficiency caused a significant decrease in glutathione concentration in copper-deficient groups compared to respective negative and copper control groups. Glutathione, an intracellular reductant responsible for maintaining the enzymes' SH groups, is detected after its synthesis in mitochondria, endoplasmic reticulum nucleus, blood plasma, and bile [46, 47].

A diminished level of glutathione indicates that cells are susceptible to oxidative stress and decreases both DNA and protein synthesis [48]. Moreover, it participates in the decomposition of H_2O_2, although the reaction is catalyzed by GSH peroxidase. Mitochondria are found plentiful in spermatids and spermatozoa and can generate reactive oxygen species under certain conditions. GSH efficiently scavenges free radicals, reactive oxygen species, and reactive nitrogen species [27, 33, 49] and its depletion in testes would enhance the vulnerability of spermatogenic cells to free radicals [50].

High GSH level was observed at the onset of spermatogenesis [51], isolated spermatogenic cells in mice [52] with low GSH level in immature hamster Sertoli cells [53]. Agarwal and Vanha – Pertulla [54] observed maximal GSH staining in the mid-piece as well as the tail region of spermatozoa.

GSH is important for sperm chromatin condensation following sperm penetration [55] and significantly impacts motility pattern [56]. Ognik *et al.,* [57] observed a decline in GSH concentration in the brain and testes in copper-deficient Wistar rats accounting for the generation of free radicals. Human spermatozoa are highly susceptible to oxidative stress because of abundant polyunsaturated fatty acids and their capacity to generate reactive oxygen species [58] and possess low concentration of antioxidant enzymes- catalase, superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione reductase [59] to neutralize ROS [60]. As observed in the study, deficiency may affect the spermatogenesis and its functions as well as the number of spermatozoa as cells with low GSH are prone to oxidative stress. Experiments revealed that the GSH effectively improves sperm morphology and motility [61, 62, 63, 64]. Moderate to low level of GSH has been reported in oligospermic, asthenozoo- and oligoasthenozoospermia men [58, 65, 66, 67].

The apparent decrement in pair-fed groups could be due to stress and starvation effect. Due to stress, the decline in GSH and free sulfhydryl groups have also been reported [33, 68]. Reactive oxygen species deleterious effects in mammalian testes are prevented mainly by extracellular SOD/SOD3, Cu/Zn-SOD/ SOD1, and Fe/Mn-SOD/ SOD2 [69].

It was further reported that SOD3 secretion by Sertoli cells may be stimulated by germ cells through cytokines [69]. Cu/Zn SOD and Mn-SOD are expressed in Leydig, peritubular myoid, and Sertoli cells [70]. Experimental evidence indicated developmental and translational regulation of SOD-2 mRNA levels with maximal levels of expression in early post-meiotic germ cells [71]. Authors [72] observed human spermatozoa are protected from increased mitochondrial superoxide radical generation by superoxide dismutase against the toxic effects of NADPH. The study reveals increased total SOD and Mn-SOD activities after copper deficiency which progressed as the duration increased, indicative of an imbalance of the redox state. Stress and starvation in the pair-fed group also exhibited an increased expression of the enzyme. A high level of total SOD and Mn-SOD in the present study can be associated with protecting the germ cells, Sertoli cells and Leydig cells against (i) enhanced superoxide radical (ii) increased generation of hydrogen peroxide and (iii) detrimental changes in seminiferous tubules after dietary copper deficiency.

Celino *et al.,* [73] showed a high level of SOD activity and Cu/Zn SOD protein concentration during early spermatogenesis. Using the immunocytochemical technique, spermatogonia exhibited strong expression for Cu/Zn SOD and weak expression in advanced-stage germ cells.

Jow *et al.,* [74] observed the highest Cu/Zn SOD mRNA level in seminiferous tubules (stages VI-VIII) prior to spermiation. SOD -/- knock-out animals exhibited an increase in oxidative stress markers [75] and reduced fertility rate *in-vitro* [76]. Garratt *et al.,* [77] reported impaired sperm motility and function impairment in Sod1-deficient mice. Dietary Cu restriction in weanling male rats revealed 68% decline in CuZnSOD activity, revealed 58% increase in superoxide anions, enhanced lipid peroxidation and damage to macromolecules [78, 79].

Decreased activity of Cu/Zn superoxide dismutase (SOD1) indicates decreased copper stores [80, 81]. Copper deficient embryos exhibited malformation and low SOD activity, accounting for increased ROS generation [82]. Ognik *et al.,*[57] observed low SOD activity in heart, lungs, and testes of copper-deficient male albino Wistar rats (Han IGS Rat [Crl: WI(Han)]) causing impairment of antioxidant defense system. The present study revealed decreased Cu-Zn SOD activity due to decreased availability of the Cu, which functions as a catalytic cofactor enhancing the vulnerability of germ cells, particularly spermatogonia being on the outside of testes barrier as well as testicular somatic cells to ROS. Catalase metabolizes hydrogen peroxide in peroxisomes; catalase bearing peroxisomes have been described in rodent Leydig cells [83].

Neinicu *et al.,*[84] reported the presence of oxidases that are responsible for generating H_2O_2 in peroxisomes in Leydig, Sertoli, and peritubular cells, besides spermatogenesis, spermatocytes and round as well as elongated spermatids. Authors [70, 85, 86] reported no activity in primary spermatocytes and round spermatids. Ognik *et al.,* [57] reported high CAT activity in albino Wistar rats (Cu-0) testes of Wistar rats fed diet having 6.5 mg copper in the standard mineral mixture. The increased catalase activity in the present study could be due to the generation of H_2O_2 as a result of the direct reduction of O_2 to counteract the harmful effect of radicals reflecting enhanced ROS and oxidative stress, which would be deleterious gonads.

CONCLUSION: Dietary copper deficiency after 2-, 4- and 6- weeks caused dysregulation of glutathione enhanced the activity of total SOD, Mn-SOD and catalase while decreasing the expression of Cu-Zn SOD. This indicates that copper homeostasis was affected, which would have a deleterious effect on testes, causing dysfunction leading to infertility- the main cause of concern.

ACKNOWLEDGEMENT: Ms. Aastha Saini thanks the University Grants Commission for the award of Junior Research Fellowship (No. 22/06/2014 (1) EU-V) and Senior Fellowship. Ms. Ankita Rajendra Kurup thanks University Grants Commission, New Delhi, for the award of the Non NET fellowship. The authors gratefully acknowledge the Department of Zoology, Centre for Advanced Studies, University of Rajasthan, Jaipur, India, for providing necessary facilities.

CONFLICTS OF INTEREST: There are no conflicts of interest between authors.

REFERENCES:

1. Shukla AK, Tiwari PK and Prakash C: Micronutrients deficiencies vis-à-vis food and nutritional security of India. Ind J Fert 2014; 10(12): 94-112.
2. Singh MV: Micronutrient nutritional problems in soils of India and improvement for human and animal health. Ind J Fert 2009; 5(4): 11-26.
3. Suttle NF: Mineral Nutrition of Livestock, Cabi Publishing, Wallingford, UK, 4th edition 2010.
4. Kohlaa S, Alic E, Amera A, Yousif T and Yassine MA: A rare case of severe copper deficiency in an infant with exclusive breastfeeding mimicking myelodysplastic syndrome . Case Rep Oncol 2020; 13: 62–68.
5. Marques AP, Riet-Correa F, Soares MP, Ortolani EL and Giuliodori MJ: Sudden deaths in cattle associated with copper deficiency. Pesquisa Vet Brasi 2003; 23(1): 21–32.
6. Kumar N, Ahlskog JE and Gross JB: Acquired hypocupremia after gastric surgery. Clin Gastroenterol Hepatol 2004; 2: 1074 –1079.
7. Rowin J and Lewis SL: Copper deficiency myel-oneuropathy and pancytopenia secondary to overuse of zinc supplementation. J Neurol Neurosurg Psychiatry 2005; 76(5): 750–751.
8. Lutsenko S, Barnes NL, Bartee MY and Dmitriev OY: Function and regulation of human copper-transporting ATPases. Physiol Rev 2007; 87: 1011–1046.
9. Kodama H, Fujisawa C and Bhadhprasit W: Inherited copper transport disorders: Biochemical mechanisms, diagnosis, and treatment. Curr Dr Met 2012; 13: 237–250.
10. Aitken RJ: Reactive oxygen species as mediators of sperm capacitation and pathological damage. Mol Reprod Develop 2017; 84: 1039–1052.
11. Trist B, Hilton JB, Crouch PJ, Hare DJ and Double KL: Superoxide dismutase 1 in health and disease: How a front-line antioxidant becomes neurotoxic. Angewandte Chemie (Int Ed. in English). 2021; 60(17): 9215–9246.
12. Aitken RJ: The Amoroso lecture. The human spermatozoon – A cell in crisis. J Repr Fer 1999; 115: 1–7.

13. Dowling DK and Simmons LW: Reactive oxygen species as universal constraints in life-history evolution. Proc Royal Soc B 2009; 276: 1737–1745.
14. Darbandi S and Darbandi M: Lifestyle modifications on further reproductive problems. Cresco J Reprod Sci 2016; 1(1): 1–2.
15. Cho ChL, Esteves SC and Agarwal A: Novel insights into the pathophysiology of varicocele and its association with reactive oxygen species and sperm DNA fragmentation. Asian J Androl 2016; 18: 186-193.
16. Du Plessis SS, Agarwal A, Halabi J and Tvrda E: Contemporary evidence on the physiological role of reactive oxygen species in human sperm function. J Assist Reprod Genet 2015; 32: 509-520.
17. Nirupama M, Devaki M, Nirupama R and Yajurvedi HN: Chronic intermittent stress-induced alterations in the spermatogenesis and antioxidant status of the testis are irreversible in albino rat. J Physiol Bioch 2013; 69: 59–68.
18. Priya PH and Reddy PS: Effect of restraint stress on lead induced male reproductive toxicity in rats. J Exp Zool A Ecol Genet Physiol 2012; 317: 455-465.
19. Valko M, Leibfritz D, Moncol J , Cronin M TD, Mazur M and Telser J: Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007; 39: 44-84.
20. Nasri H and Rafieian-Kopaei M: Protective effects of herbal antioxidants on diabetic kidney disease. J Res Med Sci 2014; 19(1): 82-83.
21. Asadi N, Bahmani M, Kheradmand A and Rafieian-Kopaei M: The impact of oxidative stress on testicular function and the role of antioxidants in improving it: A Review. J Clin Diag Res 2017; 11:IE01-IE05.
22. O'Flaherty C: Orchestrating the antioxidant defenses in the epididymis. Androl 2019; 7: 662–668.
23. Rauhala P, Andoh T and Chiueh CC: Neuroprotective properties of nitric oxide and S-nitrosoglutathione. Toxicol Appl Pharmacol 2005; 207: 91–95.
24. Gutscher M, Pauleau AL, Marty L, Brach T, Wabnitz GH, Samstag Y, Meyer AJ and Dick TP: Real-time imaging of the intracellular glutathione redox potential. Nat Methods 2008; 5: 553–559.
25. Meyer AJ and Dick TP: Fluorescent protein-based redox probes. Antioxid Redox Signal 2010; 13: 621–650.
26. Jozefczak M, Remans T, Vangronsveld J and Cuypers A: Glutathione is a key player in metal-induced oxidative stress defenses. Int J Mol Sci 2012; 13: 3145–3175.
27. Adeoye O, Olawumi J, Opeyemi A and Christiani O: Review on the role of glutathione on oxidative stress and infertility. JBRA Assist Reprod 2018; 22(1): 61-66.
28. Tapiero H, Townsend DM and Tew KD: Trace elements in human physiology and pathology. Copper. Biomed Pharmacother 2003; 57: 386-398.
29. Koval T V, Nazarova OO and Matyshevska P: Zmina vmistu glutationu v timocytah szuriv za indukcii apoptosu pid vplyvom H2O2 abo radiacii [Change in glutathione content in rat thymocytes under apoptosis induced by H_2O_2 or X irradiation]. Ukrainian Biochem J 2008; 80(2): 114–119.
30. Iskra RJ: State of glutathione component of the antioxidant system in different organs and tissues of rats under the influence of chromium nanoaquarcitate. Exp Clin Physiol Biochem 2011; 3: 28–33.
31. Chung KK: Say NO to neurodegeneration: Role of S-nitrosylation in neurodegenerative disorders. Neurosignals 2006; 15: 307–313.
32. Atakisi O, Erdogan HM, Atakisi E, Citil M, Kanici A, Merhan and Uzun M: Effects of reduced glutathione on nitric oxide level, total antioxidant and oxidant capacity and adenosine deaminase activity. Eur Rev Med Pharmacol Sci 2010; 14: 19–23.
33. Aquilano K, Baldelli S and Ciriolo MR: Glutathione: new roles in redox signaling for an old antioxidant. Front Pharmacol 2014; 5: 196 – 208.
34. Zelko I, Mariani TJ and Folz RJ: Superoxide dismutase multigene family: A comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution and expression. Free Radic Biol Med 2002; 33: 337–349.
35. Gu W, Morales, C and Hecht N B: In male mouse germ cells, copper-zinc superoxide dismutase utilizes alternative promoters that produce multiple transcripts with different translation potential. J Biol Chem 1995; 270: 236–243.
36. Beem KM, Rich WE and Rajagopalan KV: Total reconstitution of copper zinc superoxide dismutase. J Biol Chem 1974; 249: 7298–7305.
37. Nonogaki T, Noda Y, Narimoto K, Shiotani M, Mori T, Matsuda T and Yoshida O: Localization of Cu Zn-superoxide dismutase in the human male genital organ. Hum Reprod 1992; 7: 81–85.
38. Bansal AK: Manganese: A potent antioxidant in semen. Iranian J Appl Anim Sci 2013; 3(2): 217–222.
39. Sies H: Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. Redox Biol 2017; 11: 613–619.
40. Lopes IMD, de Oliveira IM, Bargi-Souza P, Cavallin MD, Kolc CSM, Khalil NM, Quinaia SP, Romano MA and Romano RM: Effects of silver nanoparticle exposure to the testicular antioxidant system during the prepubertal rat stage. Chem Res Toxicol 2019; 32: 986–994.
41. Brehne HE and Burch HB: Enzymatic assay for glutathione. Anal Biochem 1976; 74: 189-197.
42. Palamanda JR and Kehrer JP: Inhibition of protein carbonyl formation and lipid peroxidation by glutathione in rat liver microsomes. Arch Biochem Biophys 1992; 293: 103-109.
43. Marklund S and Marklund G: Involvement of superoxide anion radical in the autooxidation of pyragallol and a convenient assay of superoxide dismutase. Eur J Biochem 1974; 47: 469-474.
44. Geller BL and Winge DR: A method for distinguishing Cu-Zn- and Mn – containing superoxide dismutase. Anal Biochem 1984; 128: 86-92.
45. Sinha AK: Colorimetric assay of catalase. Anal Biochem 1972; 47: 389-394.
46. Forman H J, Zhang H and Rinna A: Glutathione: Overview of its protective roles, measurement and biosynthesis. Molec Aspects Med 2009; 30: -12.
47. Lu S C: Glutathione synthesis. Biochim Biophys Acta 2013; 1830: 3143–3153.
48. Gul M, Kutay FZ, Temocin S and Hanninen O: Cellular and clinical implications of glutathione. Ind J Exp Biol 2000; 38: 625–634.
49. Lushchak VI: Glutathione homeostasis and functions: Potential targets for medical interventions. J Amino Acids 2012; 2012: doi:10.1155/2012/736837
50. Aitken RJ: Falling sperm counts twenty years on: Where are we now. Asian J Androl 2013; 15: 204-207.
51. Calvin HI and Turner SI: High levels of glutathione attained during postnatal development of rat testis. J Exp Zool 1982; 219: 389-393.
52. Grosshans K and Calvin HI: Estimation of glutathione in purified populations of mouse testis germ cells. Biol Reprod 1985; 33: 1197-1205.

53. Den Boer PJ, Mackenbach P and Grootegoed JA: Glutathione metabolism in cultured Sertoli cells and spermatogenic cells from hamsters. J Reprod Fertil 1989; 87: 391-400.
54. Agarwal YP and Vanha-Perttula T: Glutathione, L-glutamic acid and γ-glutamyl transpeptidase in the bull reproductive tissues. Int J Androl 1988; 11(2): 123–131.
55. Kim IH, Langendonckt A Van, Soom A Van, Vanroose G, Casi AL, Hendriksen PJM and Bevers MM: Effect of exogenous glutathione on the *in-vitro* fertilization of bovine oocytes. Theriogenology 1999; 52 (3): 537-547.
56. Lenzi A, Lombardo F, Gandini L, Culasso F and Dondero F: Glutathione therapy for male infertility. Arch Androl 1992; 29: 65-68.
57. Ognik K, Tutaj K, Cholewińska E, Cendrowska-Pinkosz M, Dworzański W, Dworzańska A and Juśkiewicz J: The effect of a rat diet without added Cu on redox status in tissues and epigenetic changes in the brain. Ann Anim Sci 2020; 20 (2): 503–520.
58. Hesham N, Moemen LA and Abu Elela MH: Studying the levels of malondialdehyde and antioxidant parameters in normal and abnormal human seminal plasma. Aus J Basic Appl Sci 2008; 2: 773-778.
59. Potts RJ, Notarianni LJ and Jefferies TM: Seminal plasma reduces exogenous oxidative damage to human sperm, determined by the measurement of DNA strand breaks and lipid peroxidation. Mutat Res 2000; 447: 249-256.
60. Agarwal A and Sekhon LH: The role of antioxidant therapy in the treatment of male infertility. Hum Fertil 2010; 13: 217–25.
61. Lombardo F, Sansone A, Romanelli F, Paoli D, Gandini L and Lenzi A: The role of antioxidant therapy in the treatment of male infertility: An overview. Asian J Androl 2011; 13(5): 690-697.
62. Ahmadi S, Bashiri R, Ghadiri-Anari A and Nadjarzadeh A: Antioxidant supplements and semen parameters: An evidence based review. Int J Reprod Biomed Yazd 2016; 14(12): 729-736.
63. Majzoub A and Agarwal A: Antioxidant therapy in idiopathic oligo asthenoteratozoospermia. Ind J Urol 2017; 33(3): 207-214.
64. Majzoub A and Agarwal A: Systematic review of antioxidant types and doses in male infertility: Benefits on semen parameters, advanced sperm function, assisted reproduction and live-birth rate. Arab J Urol 2018; 113-124.
65. Ochsendorf FR, Buhl R, Bästlein A and Beschmann H: Glutathione in spermatozoa and seminal plasma of infertile men. Hum Reprod 1998; 13: 353-359.
66. Chaudhari AR, Das P and Singh R: Study of oxidative stress and reduced glutathione levels in seminal plasma of human subjects with different fertility potential. Biomed Res 2008; 19: 207-210.
67. Fafula RV, Onufrovych OK, Iefremova UP, Melnyk OV, Nakonechnyi IA, Vorobets DZ and Vorobets ZD: Glutathione content in sperm cells of infertile men. Regu Mech Biosys 2017; 8(2): 157–161.
68. Eskiocak S, Gozen AS, Yapar SB, Tavas F, Kilic AS and Eskiocak M: Glutathione and free sulphydryl content of seminal plasma in healthy medical students during and after exam stress. Hum Reprod 2005; 20(9): 2595–2600.
69. Mruk DD, Silvestrini B, Mo MY and Cheng CY: Antioxidant superoxide dismutase – a review: Its function, regulation in the testis and role in male fertility. Contraception 2002; 65: 305–311.
70. Bauché F, Fouchard MH and Jégou B: Antioxidant system in rat testicular cells. FEBS Lett 1994; 349: 392–396.
71. Gu W and Hecht NR: Translation of a testis-specific Cu/Zn superoxide dismutase (SOD-1) mRNA is regulated by a 65-kilodalton protein which binds to its 5' untranslated region. Mol Cell Biol 1996; 16: 4535–4543.
72. Armstrong JS, Bivalacqua TJ, Chamulitrat W, Sikka S and Hellstrom WJ: A comparison of the NADPH oxidase in human sperm and white blood cells. Int J Androl 2002; 25: 223-229
73. Celino FT, Yamaguchi S, Miura C, Ohta T, Tozawa Y, Iwai T and Miura T: Tolerance of spermatogonia to oxidative stress is due to high levels of Zn and Cu/Zn superoxide dismutase. PLoS ONE 2011; 6(2): e16938-e16949.
74. Jow WW, Schlegel PN, Cichon Z, Phillips D, Goldstein M and Bardin CW: Identification and localization of copper-zinc superoxide dismutase gene expression in rat testicular development. J Androl 1993; 14: 439–447.
75. Perez VI, Bokov A, Van Remmen H, Mele J, Ran QT, Ikeno Y and Richardson A : Is the oxidative stress theory of aging dead? Biochim Biophys Acta 2009; 1790: 1005–1014.
76. Tsunoda S, Kawano N, Miyado K, Kimura N and Fujii J: Impaired fertilizing ability of superoxide dismutase 1-deficient mouse sperm during *in-vitro* fertilization. Biol Reprod 2012; 87: 1-6.
77. Garratt M, Bathgate R, Graaf SPde and Brooks RC: Copper-zinc superoxide dismutase deficiency impairs sperm motility and *in-vivo* fertility. Reprod 2013; 146: 297–304.
78. Lynch SM, Frei B, Morrow JD, Roberts II LJ, Xu A, Jackson T, Reyna R, Klevay LM, Vita JA and Keaney Jr JF: Vascular superoxide dismutase deficiency impairs endothelial vasodilator function through direct inactivation of nitric oxide and increased lipid peroxidation. Arterioscler Thromb Vasc Biol 1997; 17: 2975–2981.
79. Picco SJ, De Luca JC, Mattioli G and Dulout FN: DNA damage induced by copper deficiency in cattle assessed by the Comet assay. Mutat Res 2001; 498: 1–6.
80. Milne DB and Nielsen FH: Effects of a diet low in copper on copper-status indicators in postmenopausal women. The Am J Clin Nutr 1996; 63: 358–364.
81. Reiser S, Smith JC, Mertz W, Holbrook JT, Scholfield DJ, Powell AS, Canfield WK and Canary JJ: Indices of copper status in humans consuming a typical American diet containing either fructose or starch. Am J Clin Nutr 1985; 42: 242–251.
82. Hawk SN, Lanoue L, Keen CL, Kwik-Uribe CL, Rucker RB and Uriu-Adams JY: Copper-deficient rat embryos are characterized by low superoxide dismutase activity and elevated superoxide anions. Biol Reprod 2003; 68: 896–903.
83. Reddy J and Svadoba D: Microbodies (peroxisomes) in the interstitial cells of rodent testes. Lab Invest 1972; 26: 657 - 665.
84. Nenicu A, Luers GH, Kovacs W, David M, Zimmer A, Bergmann M and Baumgart –Vogt E: Peroxisomes in human and mouse testis: Differential expression of peroxisomal proteins in germ cells and distinct somatic cell types of the testis. Biol Reprod 2007; 77: 1060–1072.
85. Yoganathan T, Eskild W and Hansson V: Investigation of detoxification capacity of rat testicular germ cells and Sertoli cells. Free Radic Biol Med 1989; 7: 355–359.
86. Yoganathan T, Oyen O, Eskild W, Johnson T and Hansson V: Cellular localization and age-dependent changes in mRNA for glutathione –S – transferase P in rat testicular cells. Biochem Int 1989; 19: 667-672.

How to cite this article:
Saini A, Kurup AR and Nair N: Glutathione and antioxidant study in testes of Wistar rats after dietary copper deficiency. Int J Pharm Sci & Res 2022; 13(4): 1747-54. doi: 10.13040/IJPSR.0975-8232.13(4).1747-54.