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Evaluation of effects of *portulacaoleracea* extract on histopathological changes of testis tissue, following long term administration of copper sulfate in Rat

Yadollah Badakhshan[*1], Manouchehr Yousefi[2] and Reza Mirzaei[3]

[1]University of Jiroft (Iran), Agriculture faculty- department of animal science-
[2]Department of Animal Science, Faculty of Agriculture, Higher Educational Complex of saravan, Saravan, Iran
[3]Medical University of Jiroft
**Corresponding author: y.badakhshan@ujiroft.ac.ir*

ABSTRACT

This study was set to investigate whether the adverse effects of long term copper (Cu)consumption on testicular tissue could be prevented by portulacaoleracea extract administration. Twenty four mature male Wistar Rat were randomly allocated to either control or three treatment groups. The first group received distilled water 0.25 ml by gavage. (control group, n=6). The second treatment group received copper sulfate(0.25ml of a 200 mg/Kg body weight) once a day (Cu experimental group, n=6). The third treatment group was given combined treatment of copper sulfate (200 mg/kg) by gavage and water that include portulacaoleracea extract at a dose of 400ppm. (treatment group, n=6). The fourth group received distilled water 0.25 ml by gavage and had availability to water that ncludeportulacaoleracea extract at a dose of 400ppm. (group, n=6). each group were sacrificed after 4 weeks from the beginning of treatments. Testes were removed for histopathological and histomorphometrical evaluations. Morphometrically, the mean of seminiferous tubules diameter, sertoli cells diameter, epithelial height, Meiotic index and the percentage of spermatogenesis in Cu groups showed significant decrease than those of the control groups ($p<0.05$). A recovery was observed in these parameters in groups have received extract. There was statistically non difference between control and third ,fourth treatment groups. A recovery was observed in these parameters in treatment groups compared to the control group ($p<0.05$). Results showed that long term administration of cu leads to histological impairments of testis and portulacaoleracea extract supplementation would offset these damaging effects.

Key words: portulacaoleracea extract, Histopathology, Copper sulphate poisoning, Rat, Testes

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INTRODUCTION

Characterization of the antioxidant activity of vegetables may also yield more insight into their functionality. Dietary antioxidants are necessary to cope with reactive oxidant species that could damage DNA, RNA, modify proteins, and cause lipid peroxidation of cellular targets. Antioxidants may inhibit the initiation or propagation of oxidation[1-3].Vegetable extracts such as Portulacaoleracea have high antioxidant activity may also be useful for human consumption [3] .

Vegetables may confer a variety of health benefits. Increased vegetable consumption is correlated with reduced risks of cardiovascular disease, stroke, liver protective effects, arthritis, inflammatory bowel diseases, and some cancers, Anti-aging Effect [4, 5] .

Numerous studies have demonstrated that plant-derived natural compounds exhibit protective activities against genotoxicity caused by oxidative stress[1], and there is particular interest in natural substances identified from herbal compounds. Portulacaoleracea L. (Portulacaceae) is listed by the World Health Organization as one of the most commonly used medicinal plants. In folk medicine, *P.oleracea* is utilized as an antipyretic, anti-scorbutic, antiseptic, antispasmodic, diuretic, anthelmintic, and for treatment of urinary disorders[6]. The aerial parts of this plant are used medicinally for reducing pain and swelling[7]. Recent pharmacological studies have also shown muscle relaxant activity[8], reduction in locomotor activity, an increase in the onset time of pentylenetetrazole-induced convulsion [9], gastroprotective action, and analgesic, anti-inflammatory effects, and antioxidant properties[10]. For example, *P.oleracea* has an inhibitory effect on lipopolysaccharide(LPS) and interferon-g (IFN-g) induced NO production[1, 11]. Zidan et al (2014) showed that Portulacaoleracea reduces triglyceridemia, cholesterolemia, and improves lecithin: cholesterol acyltransferase activity in rats fed enriched-cholesterol diet [12]. A hypoglycemic effect of *P. oleracea* without any genotoxic activity was also reported[13, 14]. Furthermore, *P. oleracea* is a rich source of omega-3 fatty acids, gallotannins, kaempferol, quercetin, apigenin, and glutathione[15]. However, little is known of the anti-histological and anti-morphological changes properties of *P. oleracea*. normal spermatogenesis, sperm maturation, DNA metabolism and repair and gene expression in germ cells need to normal concentration of antioxidants, nutrients and elements and infertility of male sex has been related to their deficiency[16-18]. Male and female infertility has positive correlation with reactive oxygen species concentration[19] and has been implicated in gonadal dysfunction, a decreased testicular weight and shrinkage of the seminiferous tubules[20, 21].

In the present study, we examined the anti-oxidative stress effects of aqueous extracts of *P. oleracea* on testis tissue, following long term administration of copper sulfate in Rat.

MATERIALS AND METHODS

Aerial parts of *P. oleracea* were collected from a cultivation located in University of Jiroft lands. The aerial parts of *P. oleracea* were cleaned, dried in the dark, and powdered with a mechanical grinder. The juice was weighed and dried under 45 C˚ in a Avon for 72 hours. Dried extract was used for making a 400 PPM solution.

Animal

Experimental groups consisted of 6 Male Wistar Rats (180–220 g) per group in four groups. They were housed at 21±1 ◦C under a 12-h light/12-h darkcycle and had free access to standard pellet diet (Javaneh Khorasan Co) and tap water. After two week adaptation. Experimental groups were consisted of control, 400 ppm extract of P. oleracea, 400 ppm extract of *P. oleracea* plus 200ppm copper sulphate and 200ppm copper sulphate group treatments. The solutions were fed orally with a gavage syringe daily for 30 days. Copper sulphate fed in a 0.25 mL from a 200ppm solution and 1 mL from 400 ppm of *P. oleracea* solution. Also control group received a 1.25 mL distillated water orally per day.

After four weeks, rats were food deprived for 12 h, anaesthetized with sodium pentobarbital (60 mg/kg body weight) and euthanized with an overdose. They were decapitated and testis collected and stored in 10% formalin. All testis were embedded in parafine wax and cut in 5 micrometer thickness stained haematoxylin and eosin and examined with a expert pathologist under light microscope. The mean seminiferous tubules diameter, epithelial height, Meiotic index and the percentage of spermatogenesis in each testis were measured.

RESULTS

Spermatogenesis and meiotic index

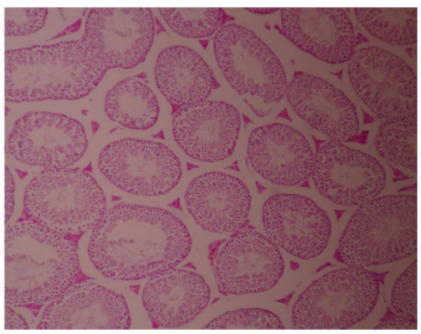
The mean percentage of Spermatogenesis and meiotic index of treatments are represented in table 1. mean percentage of Spermatogenesis and meiotic index of control, 400 ppm extract of *P. oleracea*, 400 ppm extract of *P. oleracea* plus 200ppm copper sulphate and 200ppm copper sulphate group treatments were 77.16± 0.91,72.5± 0.94,73.8± 0.84,58.81± 0.70 and3.2± 0.03, 2.9 ± 0.04, 2.8 ± 0.04, 1.64 ± 0.07 respectively. These parameters reduced significantly in copper sulphate treatment group significantly ($P< 0.05$) but there wasn't any significant difference between control and *P. oleracea* extract treated groups.

Seminiferous tubules diameter and epithelial height

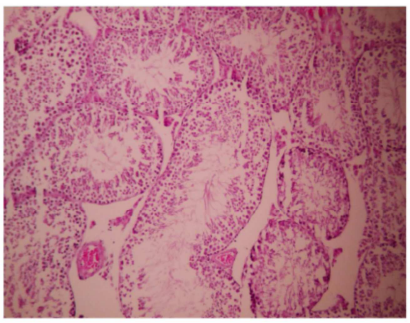
The mean Seminiferous tubules diameter and epithelial height of control, 400 ppm extract of P. oleracea, 400 ppm extract of *P. oleracea* plus 200ppm copper sulphate and 200ppm copper sulphate group treatments were 220 ± 4,217 ± 4.8, 218± 4.1, 196±3.6 µm and 60.2 ±1.5, 58± 1.8, 55±1.4, 43±3.6 µm. *P. oleracea* treatment groups had similar Seminiferous tubules diameter and epithelial height. But copper sulphate treatment group had significantly reduction in these parameters (P< 0.05).

Table 1. mean ± SEM of morphological and histological parameters of rat. Different letters in column are significantly different (P< 0.05)

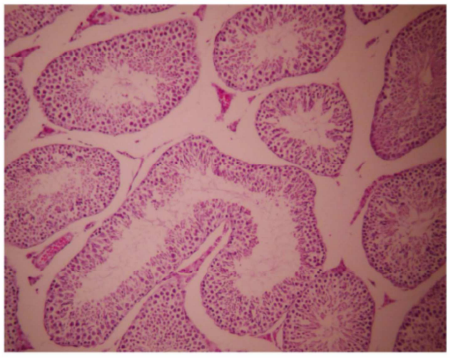
Treatment group	Spermatogenesis percentage	meiotic index	Seminiferous tubules diameter (µm)	epithelial height (µm)
Control	77.16± 0.91[a]	3.2± 0.03[a]	220 ± 4[a]	60.2 ±1.5[a]
P. oleracea extract	72.5± 0.94[a]	2.9 ± 0.04[a]	217 ± 4.8[a]	58± 1.8[a]
P. oleracea extract + copper sulphate	73.8± 0.84[a]	2.8 ± 0.04[a]	218± 4.1[a]	55±1.4[a]
copper sulphate	58.81± 0.70[b]	1.64 ± 0.07[b]	218± 4.1[b]	43±3.6[b]



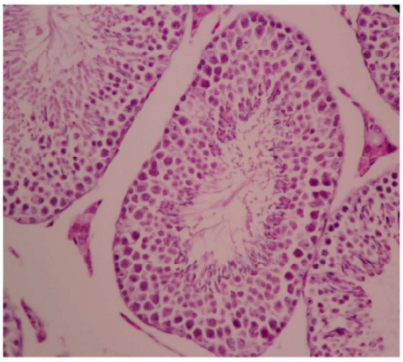
Picture 1. Control Group



Picture 2. Copper sulphate Group



Picture 3. P. oleracea extract Group



Picture 4. P. oleracea extract + Copper sulphate Group

Photos show morphological and histological of different groups compared to control group. Damaged seminiferous tubules of copper sulphate group (picture 2) and normal morphological pictures of three other groups are shown here.

DISCUSSION

The results of the recent study demonstrated curing effects of *P. oleracea* extract treatment on damages of copper sulphate poisoning in rat testis.

Copper poisoning induce oxidative stress in body organs, consequently reactive oxygen species production demolish antioxidant defense capacity of the cell[21]. That may lead to adverse biological results such as damage to proteins, lipids structures of cell especially proliferative cell such as sex gonad cells[1].

Damaged spermatogenesis and meiotic index has been demonstrated in rodent with copper sulphate poisoning. Several researcher reported copper sulphate poisoning as a method to induce oxidative stress in body organs[21].

herbs are claimed to have high antioxidant properties as well as medicinal properties. The complex mixture of phytochemicals in vegetables and fruits provide overlapping or complementary effects that contribute to the protective effect of health [2, 3, 6]. Several studies have shown a positive effects of Portulacaoleracea (Portulacaceae family) on diabetic, hypoxic, fatty liver disease[10, 12-14].

The water extracts of *P. oleracea* show no cytotoxicity or genotoxicity and have been certified safe for daily consumption as a vegetable[4]. The aqueous extract of *P. oleracea* displayed protective activities against H2O2-induced chromosomal damage, while the ethanolic extract did not[1]. The antioxidant activities of *P. oleracea* extracts have been previously demonstrated, and are suggested to be related to its constituents, such as omega-3 fatty acids, gallotannins, kaempferol, quercetin, apigenin, a-tocopherols, flavonoids (quercetin), ascorbic acid, and glutathione[9, 10, 15, 22] . Ascorbic acid and glutathione can scavenge free radicals and block the peroxidation of nucleic acids and DNA damage, as well as reduce the production of free radicals[1].

Similarly protective effects of aqueous extract of *P. oleracea* has been shown in our experiment. As reportedno negative effect of *P. oleracea* extract in researches . Not only There wasn't any damaged morphological parameters of testis in 400 ppm treated group. But also administration of P. oleraceaExtract prevent morphological and histological changes in copper sulphate treated group.

When aqueous extract of *P. oleracea* add to human lymphocyte culture, significantly DNA impairments was reduced[1].

As reported by Zidan et al 2014, Portulacaoleracea reduces triglyceridemia, cholesterolemia, and improves reverse cholesterol transport in rat fed enriched-cholesterol diet. *P. oleracea* extract treated rat have had significantly lower cholesterol and triglyceride in their liver tissue[5, 12].

observations and results indicated that the fresh crude extract of Portulacaoleracea significantly stimulated wound contraction[7]. Accordingly to our results, morphological and histological tissue of control and Portulacaoleracea extract received groups was similar.

CONCLUSION

In this study healing effect and antioxidant activities of Portulacaolerecea extract on copper sulphate poisoning of rat was observed. There was no significant difference between histological and morphological parameters of control and oleracea extract received groups. More study about of anti-oxidative stress and oleracea extract consumption required.

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REFERENCES

[1] J. Behravan;F. Mosafa;N. Soudmand;E. Taghiabadi;B.M. RazaviG. Karimi. *Journal of acupuncture and meridian studies*.**2011 4** (3) 193-197.
[2] J.F.O. Costa;A.C. Kiperstok;J.P.d.L. David;J.M. David;A.M. Giulietti;L.P. de Queiroz;R.R. dos SantosM.B.P. Soares. *Fitoterapia*.**2007 78** (7) 510-514.
[3] Y. Lim E. Quah. *Food chemistry*.**2007 103** (3) 734-740.
[4] N. Guangcai;Z. Dan;J. YingziP. Yongquan. *Chinese Agricultural Science Bulletin*.**2006 10** 008.
[5] M. Anusha;M. Venkateswarlu;V. Prabhakaran;S.S. Taj;B.P. KumariD. Ranganayakulu. *Indian journal of pharmacology*.**2011 43** (5) 563.

[6] L. Xiang;D. Xing;W. Wang;R. Wang;Y. DingL. Du. *Phytochemistry*.**2005 66** (21) 2595-2601.
[7] A. Rashed;F. AfifiA. Disi. *Journal of Ethnopharmacology*.**2003 88** (2) 131-136.
[8] F. Malek;M. Boskabady;M. BorushakiM. Tohidi. *Journal of ethnopharmacology*.**2004 93** (1) 57-62.
[9] K. Chan;M. Islam;M. Kamil;R. Radhakrishnan;M. Zakaria;M. HabibullahA. Attas. *Journal of ethnopharmacology*.**2000 73** (3) 445-451.
[10] C.-J. Chen;W.-Y. Wang;X.-L. Wang;L.-W. Dong;Y.-T. Yue;H.-L. Xin;C.-Q. LingM. Li. *Journal of ethnopharmacology*.**2009 124** (2) 246-250.
[11] H. Shen;G. Tang;G. Zeng;Y. Yang;X. Cai;D. Li;H. LiuN. Zhou. *Carbohydrate polymers*.**2013 93** (2) 395-400.
[12] Y. Zidan;S. Bouderbala;F. Djellouli;M. Lacaille-DuboisM. Bouchenak. *Phytomedicine*.**2014 21** (12) 1504-1508.
[13] J.-f. Gu;Z.-y. Zheng;J.-r. Yuan;B.-j. Zhao;C.-f. Wang;L. Zhang;Q.-y. Xu;G.-w. Yin;L. FengX.-b. Jia. *Journal of ethnopharmacology*.**2015 161** 214-223.
[14] M.-I.K. El-Sayed. *Journal of ethnopharmacology*.**2011 137** (1) 643-651.
[15] S. Ercisli;I. Coruh;A. GormezM. Sengul. *Italian J. Food Sci*.**2008 20** (4) 533.
[16] A. García-Contreras;Y. De Loera;C. García-Artiga;A. Palomo;J.A. Guevara;J. Herrera-Haro;C. López-Fernández;S. JohnstonJ. Gosálvez. *Reproductive Toxicology*.**2011 31** (4) 570-573.
[17] S. Pesch;M. BergmannH. Bostedt. *Theriogenology*.**2006 66** (2) 307-313.
[18] A.H. Colagar;E.T. MarzonyM.J. Chaichi. *Nutrition Research*.**2009 29** (2) 82-88.
[19] U. Luderer, *Chapter Four - Ovarian Toxicity from Reactive Oxygen Species*, in *Vitamins & Hormones*, Gerald, L., Editor. **2014**, Academic Press. p. 99-127.
[20] O. Akinloye;F.M. Abbiyesuku;O.O. Oguntibeju;A.O. ArowojoluE.J. Truter. *Reproductive biology*.**2011 11** (2) 83-97.
[21] H. Babaei;R. KheirandishL. Ebrahimi. *Asian Pacific Journal of Tropical Biomedicine*.**2012 2** (3) S1615-S1619.
[22] S.F. Arruda;E.M. SiqueiraE.M. Souza. *Annals of nutrition and metabolism*.**2004 48** (4) 288-295.