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## ORIGINAL ARTICLE

### Effects of Curcumin Nanoparticles on the Tissue Oxidative Stress Following Testicular Torsion and Detorsion in Rat Model

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#### Keywords:

Ischemia-reperfusion;

Curcumin nanoparticle;

Testicular torsion.

#### Abstract

**Objective-**The aim of the present study was to investigate the effects of curcumin nanoparticles (CNP) on stress oxidative following experimental ischemia-reperfusion injury in the rat testes.

**Design-**Experimental Study

**Animals-** Seventy-seven healthy male Wistar rats

**Procedures-**The animals weighing approximately 250 g were randomly assigned to four experimental groups (n = 18): Ischemia: torsion group (created by 720° rotation of testis on both sides for 2 h). Ischemia/Reperfusion (I/R): torsion for 2 h followed distortion, CNP 10: received 10 mg/kg IP administration of CNP 30 min before surgery then remaining testes were twisted and untwisted, CNP 20: received 20 mg/kg IP administration of CNP 30 min before surgery then remaining testicles were twisted and untwisted. Unilateral orchiectomy of left or right testicles was performed on days 0 and 12 with immediately sampling. Some twisted then untwisted testicles were remained and sampled 60 days after surgery. An additional group was considered (n=5) to be sampled without any operation as control group. The samples of testicular tissue homogenates were taken on Days 0, 12 and 60, and their liquid extracts were collected and assayed for Superoxide dismutase (SOD), Malondialdehyde (MDA) and Glutathione peroxidase (GPX). The effect of treatment and day of sampling on the variables was analyzed using Two-Way ANOVA.

**Results-** The results of the study showed an ameliorated balance of GPX, SOD and MDA following testicular torsion in this study. The CNP treated animals (with both administered doses) showed significantly improved the balance of the enzymes compared to untreated animals ( $p < 0.0001$ ).

**Conclusion and clinical relevance-** In conclusion, IP administration of CNP may be helpful in minimizing oxidative stress related enzymes following testicular ischemia-reperfusion in rat.

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## 1. Introduction

The torsion of the spermatic cord is an emergency case problem in human that results from rotation of the testis and epididymis around the axis of the spermatic cord. Urgent operative interventions are required to re-establish the blood flow and circumvent the perpetual damaging effects on the testis which may turn out to be decreased spermatogenesis in majority of cases thus permanently lowering fertility chances.<sup>1</sup>

Reperfusion of the ischemic tissue leads to much more serious damage to the tissue than the damage caused by ischemia.<sup>2</sup> Reperfusion-related damage in the cell is created by many factors, mostly including oxygen-derived free radicals, which are rapidly generated in the tissue as a result of reperfusion.<sup>3</sup> Due to physiological or pathological alterations, oxidative damage takes place with changes in favor of the oxidation process.<sup>4</sup> Prompt diagnosis to reduce ischemic and reperfusion injury, and its consequents are still inevitable with this approach. Therefore, studies on preventing reperfusion injury seem very important.<sup>5</sup>

A proposed pathogenesis of tissue injury during reperfusion is accumulation of the activated neutrophils that release reactive oxygen species.<sup>6</sup> Lipid peroxidation in the cell is the most deleterious effects of free radicals that end up reduction in the membrane potential and subsequently, cell injury. Malondialdehyde (MDA), one of the end products of lipid peroxidation, also results in serious cell damage through induction of polymerization and cross linking in membrane components.<sup>7</sup> Oxygen free radicals react with DNA and form 8-hydroxyguanine (8-OHGua) that is one of the damage products of DNA.<sup>8</sup> In spite of the fact that generation of free oxygen radicals occurs continuously in cells, the presence of endogenous antioxidant defense systems preserves tissues from the harmful effects of free oxygen radicals.<sup>9</sup> Various agents, anti-inflammatory and antioxidant free radical scavengers have been reported with promising beneficial effects on prevention of ischemic/reperfusion injuries in tissues.<sup>10, 11</sup>

Curcumin is the main phenolic pigment extracted from turmeric, the powdered rhizome of *Curcuma longa*, along with demethoxy curcumin and bisdemethoxy curcumin.<sup>12</sup> Extensive research indicates that curcumin possesses potent antioxidant, anti-inflammatory, properties, and it also inhibits lipid peroxidation and scavenges superoxide anion, singlet oxygen, nitric oxide, and hydroxyl radicals.<sup>13, 14</sup> Administration of curcumin has been reported to be effective in reversing tissue damage induced by ischemia reperfusion injury in ovarian torsion.<sup>15</sup>

Curcumin, a naturally occurring polyphenolic compound, is a considerably promising compound, however, its poor water solubility and fast degradation profile make it compromise over its bioavailability way below the threshold level on administration. Over a period of time, a lot of emphasis has been given to improve the biodistribution of native curcumin, but it is only recently that the application of the field of nanotherapeutics has significantly improved its therapeutic efficacy. This is through the development of nanorange formulations of curcumin, popularly known as the nanocurcumin.<sup>16</sup>

The present study aimed to study peritoneal effects of curcumin nanoparticles (CNP) on the oxidative stress parameters following ischemia/reperfusion injury.

## 2. Materials and Methods

### Materials

Materials in this experiment were purchased from Merck Germany (KH<sub>2</sub>PO<sub>4</sub>, EDTA, GSH, B-NADPH, NaN<sub>3</sub>) and Sigma USA (curcumin, nitroblue tetrazolium, KCl, 2-thiobarbiturate, acetic acid, sodium lauryl sulfate, n-butanol:pyridine). Other materials were specified throughout the text.

### Study design and animals

The animals were maintained at 28±1° C, relative humidity 60%, natural light/dark cycle, and provided with standard

food pellets (diet composition, wheat broken-moisture 9.0%, crude protein 11.5%, crude fat 1.9%, crude fiber 4.0%, Ash 0.2%, nitrogen-free extract 73.4%) and tap water *ad libitum*.

Seventy-seven healthy male Wistar rats weighing approximately 250 g were randomly assigned to four experimental groups (n = 18): Ischemia: torsion group (created by 720° rotation of testis on both sides for 2 h). Ischemia/Reperfusion (I/R): torsion for 2 h followed distortion, CNP 10: received 10 mg/kg IP administration of CNP 30 min before surgery then remaining testis were twisted and untwisted, CNP 20: received 20 mg/kg IP administration of CNP 30 min before surgery then remaining testicles were twisted and untwisted. Unilateral orchiectomy of right/left testicles was performed on days 0 and 12 with immediate sampling. The twisted then untwisted testicles were remained and sampled 60 days after surgery. To get the normal ranges of the biochemical values, an additional group was considered (n = 5) to be sampled without any operation as control group. The samples of testicular tissue homogenates were taken on days 0, 12, and 60, and their liquid extracts were collected and frozen in -80° C before assessment.

### **Preparation of CNP**

The CNP were prepared based on the previously described method<sup>17</sup>. In brief, curcumin (100 mg, 0.27 mmol) was taken in dichloromethane (20 ml), and 1 ml of this solution was sprayed into boiling water (50 ml) dropwise with a flow rate of 0.2 ml/min in 5 min under ultrasonic conditions, with an ultrasonic power of 100 W and a frequency of 30 kHz. After sonication for 10 min, the contents were stirred at 200-800 rpm at room temperature for about 20 min when a clear orange-colored solution was obtained.

The solution was concentrated under reduced pressure at 50° C and then freeze-dried to obtain an orange powder. A

co-TLC of the powdered sample with standard CNP showed both to have the same Rf values. Further, <sup>1</sup>H nuclear magnetic resonance NMR and ultraviolet (UV) spectra of the lyophilized powder confirmed it to be CNP. Further, maintaining the drop flow was significant for both the formation of nanoparticles and maintaining uniformity in their size.

The mean particle diameter of CNP was measured by dynamic light scattering (DLS) performed on Malvern Zetasizer S90 series. The sample was prepared by taking 1 mg of the lyophilized CNP powder in 10 ml of distilled water. The sample was prepared by placing a drop of the aqueous dispersion of CNP on the copper grid and allowing it to air dry.<sup>17</sup>

### **Surgical procedure**

Animals were anesthetized by IP administration of ketamine-xylazine (ketamine 5%, 90 mg/kg and xylazine 2%, 5 mg/kg, Alfasan, the Netherlands). The procedure was carried out based on the guidelines of the Ethics Committee of the International Association for the Study of Pain.<sup>18</sup> The ethical Committee of the University approved all the experiments.

Each testis was exteriorized through a low midline laparotomy, the gubernaculum was divided and the testis was freed from the epididymo-testicular membrane (Figure 1). The testis was twisted 720° clockwise and maintained by fixing the testis to the adjacent abdominal wall with a 4-0 nylon suture placed through the tunica albuginea. The testis was kept wet using sterile normal saline soaked gauze. At the appropriate time, three hours of ischemia, the testis was counter-rotated to the normal position, the gubernaculum was rejoined, and the testis was reinserted into the scrotum via the inguinal canal.



**Figure 1.** Each testis was exteriorized through a low midline laparotomy, the gubernaculum was divided and the testis was freed from the epididymo-testicular membrane and twisted 720° clockwise.

### *Biochemical assessments within tissues*

Samples of liquid extracts from the tissues were prepared according to the previous study. Briefly, the tissue samples of testes were kept at  $-80^{\circ}\text{C}$  for 3 days, and then enzyme activities were determined in the tissues of rat testes. The testes tissues were ground with liquid nitrogen in a mortar. One half gram was weighed for each group and then treated with 4.5 ml of an appropriate buffer. This mixture was homogenized on ice with use of an ultra-turrax homogenizer (IKA, Werke, Germany) for 15 minutes. Homogenates were filtered and centrifuged by using a refrigerator centrifuge at  $4^{\circ}\text{C}$ . Then the supernatants were used to determine the enzymatic activities. All assays were carried out at room temperature.<sup>19</sup>

### *Superoxide dismutase assay*

Superoxide dismutase (SOD) was estimated by assessment of the generation of superoxide radicals produced by xanthine and the xanthine oxidase system, which reacts with nitroblue tetrazolium to form formazan dye using special kit (Navandsalamat, Iran).<sup>20</sup> Superoxide dismutase activity was then measured at 560 nm wave length by the degree of inhibition of this reaction and is expressed as mmol per minute per mg of tissues.

### *MDA assay*

MDA levels were measured using special kit (Navandsalamat, Iran). The lipid peroxidation was determined by estimating MDA using the thiobarbituric acid test.<sup>21</sup> The testicles were weighed digested and homogenized in 10 ml of 100 g/l KCl. The homogenate (0.5 ml) was added to a solution containing 2-thiobarbiturate (1.5 ml of 8 g/l), acetic acid (1.5 ml of 200 g/l), sodium lauryl sulfate (0.2 ml of 80 g/l), and distilled water (0.3 ml).

The mixture was incubated at  $98^{\circ}\text{C}$  for 1 hr. n-butanol:pyridine 5 ml (ratio: 15:1) was then added. The mixture was vortexed for 1 min and centrifuged for 30 min at 4000 rpm. The absorbance of the supernatant was measured at 532 nm wave length using a spectrophotometer. The standard curve was obtained by using 1,1,3,3-tetramethoxypropane.

### *Glutathione peroxidase assay*

Glutathione peroxidase (GPX) activity within the tissues was determined according to the method of Lawrence and Burk using kit (Navandsalamat, Iran).<sup>22</sup> Following tissue homogenization, supernatant was used for GPX measurement. Following the addition of  $\text{KH}_2\text{PO}_4$ , EDTA, GSH, B-NADPH,  $\text{NaN}_3$ , and GR, the mixture was incubated. As soon  $\text{H}_2\text{O}_2$  was added the chronometer was turned on and the absorbance at 340 nm wave length was recorded for 5 min every 15 sec.

### *Statistical analysis*

The effects of treatments and time of sampling on the values of enzyme activities were analyzed using two-way ANOVA in SAS 9.1.3 (SAS Inc, Chicago, IL, US) and *pdiff* post hoc test was used to compare the means. Data were expressed as least square mean and standard error (SE) of means. The *p* values less than 0.05 considered as significant.

### 3. Results of SID

Tables 1-3 show the results of the study. Data analysis showed a significant effect of treatments on all three enzymes activity ( $p < 0.0001$ ). Two-way interaction of time of sampling and group of treatment was also significantly different ( $p < 0.0001$ ) among groups. Based on the tables, two levels of CNP, effectively recovered tissue enzymes activities, however, the values were not reached to normal levels ( $p > 0.05$ ).

### 4. Discussion

The present study it was investigated whether interaperitoneal administration of curcumin nanoparticles was useful or not in the prevention of testicular damage in ischemia/reperfusion conditions in rat and it was found to have beneficial effects. In the present study, levels of SOD, MDA and GPX in testicular tissue were assessed and compared in all the experimental groups. Our results showed that in the CNP 10 and CNP 20 groups, SOD was

**Table 1.** Superoxide dismutase levels (mmol/min/mg) in the testes from different groups at different days of sampling (mean  $\pm$  SE)

	Control (n=5)	Day of sampling		
		0 (n=18)	12 (n=18)	60 (n=18)
<b>Ischemia</b>		34.01 $\pm$ 0.87 <sup>Ab</sup>	35.00 $\pm$ 0.87 <sup>Ab</sup>	<b>68.68 <math>\pm</math> 0.87<sup>Ac</sup></b>
<b>Ischemia/Reperfusion</b>	65.8 $\pm$ 0.95 <sup>a</sup>	64.7 $\pm$ 0.87 <sup>Ba</sup>	65.28 $\pm$ 0.87 <sup>Ba</sup>	<b>68.61 <math>\pm</math> 0.87<sup>Ab</sup></b>
<b>CNP 10</b>		70.96 $\pm$ 0.87 <sup>Cb</sup>	73.05 $\pm$ 0.87 <sup>Cc</sup>	<b>66.43 <math>\pm</math> 0.87<sup>Aab</sup></b>
<b>CNP 20</b>		73.20 $\pm$ 0.87 <sup>Ca</sup>	71.65 $\pm$ 0.87 <sup>Ca</sup>	<b>66.25 <math>\pm</math> 0.87<sup>Ab</sup></b>

At least one common letter in each row (abc) and one common capitalized letters (ABC) in each column indicate no significant difference ( $p > 0.05$ ).

**Table 2.** Malondialdehyde levels ( $\mu$ mol/mg protein) in the testes from different groups at different days of sampling (mean  $\pm$  SE)

	Control (n=5)	Day of sampling		
		0 (n=18)	12 (n=18)	60 (n=18)
<b>Ischemia</b>		12.98 $\pm$ 0.21 <sup>Ab</sup>	14.18 $\pm$ 0.21 <sup>Ac</sup>	<b>6.06 <math>\pm</math> 0.21<sup>Ad</sup></b>
<b>Ischemia/Reperfusion</b>	5.0 $\pm$ 0.23 <sup>a</sup>	10.58 $\pm$ 0.21 <sup>Bb</sup>	11.33 $\pm$ 0.21 <sup>Bc</sup>	<b>6.21 <math>\pm</math> 0.21<sup>Ad</sup></b>
<b>CNP 10</b>		7.35 $\pm$ 0.21 <sup>Cb</sup>	8.31 $\pm$ 0.21 <sup>Cc</sup>	<b>7.05 <math>\pm</math> 0.21<sup>Bcb</sup></b>
<b>CNP 20</b>		7.05 $\pm$ 0.21 <sup>Cbc</sup>	7.53 $\pm$ 0.21 <sup>Dc</sup>	<b>6.85 <math>\pm</math> 0.21<sup>Bb</sup></b>

At least one common letter in each row (abc) and one common capitalized letters (ABC) in each column indicate no significant difference ( $p > 0.05$ ).

**Table 3.** Glutathione peroxidase levels (U/mg protein) in the testes from different groups at different days of sampling (mean  $\pm$  SE)

	Control (n=5)	Day of sampling		
		0 (n=18)	12 (n=18)	60 (n=18)
<b>Ischemia</b>		12.5 $\pm$ 0.21 <sup>Ab</sup>	12.81 $\pm$ 0.21 <sup>Ab</sup>	<b>37.93 <math>\pm</math> 0.21<sup>Aa</sup></b>
<b>Ischemia/Reperfusion</b>	37.6 $\pm$ 0.24 <sup>a</sup>	3.36 $\pm$ 0.21 <sup>Bb</sup>	22.8 $\pm$ 0.21 <sup>Bb</sup>	<b>37.28 <math>\pm</math> 0.21<sup>Ba</sup></b>
<b>CNP 10</b>		31.18 $\pm$ 0.21 <sup>Cb</sup>	31.73 $\pm$ 0.21 <sup>Cb</sup>	<b>35.7 <math>\pm</math> 0.21<sup>Cc</sup></b>
<b>CNP 20</b>		31.18 $\pm$ 0.21 <sup>Db</sup>	31.73 $\pm$ 0.21 <sup>Cc</sup>	<b>37.33 <math>\pm</math> 0.21<sup>ABa</sup></b>

At least one common letter in each row (abc) and one common capitalized letters (ABC) in each column indicate no significant difference ( $p > 0.05$ ).

increased compared to those in Ischemia and I/R groups and IP administration of CNP, secured testicular tissue against ischemia-reperfusion injury. MDA levels in the present study were found to be much lower in the CNP 10 and CNP 20 animals compared to those in other experimental groups. A significant increase in GPX activity was observed in testicular tissues of CNP 10 and CNP 20 animals.

There are many studies in the literature about the improvement of ischemia reperfusion injury. Studies demonstrated that the agents with antioxidant or anti-inflammatory activities may be beneficial in reducing testicular ischemia reperfusion injury.<sup>23,24</sup> Also, the beneficial effect of controlled reperfusion in the prevention of testicular tissue damage have been proved<sup>25</sup>. Essentially, early diagnosis and treatment of testicular torsion plays an important role to provide urgent protection against life-threatening complications from ischemia and to prevent future infertility.<sup>26</sup>

Curcumin has been reported as a useful agent both for the prevention and treatment of I/R injury in many organs. These protective effects are mainly believed to be based on inhibitory actions of curcumin on disease-mediated induction of inflammatory transcription factors, protein kinases, adhesion molecules, oxidative stress (OS) and inflammation.<sup>27</sup> The administration of curcumin has reported to reduce the generation of reactive oxygen species (ROS), monocyte adhesion, phosphorylation of c-Jun N-terminal kinase (JNK), p38 MAP kinase, and signal transducer and activator of transcription (STAT)-3 in TNF- $\alpha$ -stimulated cells.<sup>27</sup> It has also been documented that the administration of curcumin prior to the conservative surgery (detorsion) provides a significant decrease for the OS markers in the ovarian tissues.<sup>15</sup> The comparison between the oxidative status and antioxidative status is clear enough to suggest that the administration of curcumin, as reported previously, leads to a decrease in the OS.<sup>17</sup>

In conclusion, the findings of the present study showed that

CNP in both concentrations, 10 and 20 mg/kg, gave rise to significant improvements in OS enzymes following ischemia-reperfusion injury in testicular tissues in rats.

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### Conflict of interest

None.

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## چکیده

تأثیر نانوذرات کورکومین بر استرس اکسیداتیو بافت به دنبال پیچ خوردگی تجربی بیضه و اصلاح آن  
در مدل موش صحرایی

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**طرح مطالعه -** مطالعه آزمایشگاهی

**حیوانات -** ۷۷ موش صحرایی نر نژاد ویستار.

**روش کار -** حیوانات سالم با وزن تقریبی ۲۵۰ گرم به صورت تصادفی به ۴ گروه تجربی (۱۸ تایی) تقسیم شدند: گروه ایسکمی: پیچ خوردگی (با پیچاندن ۷۲۰ درجه بیضه‌های هر دو سمت) به مدت ۲ ساعت، ایسکمی/پرفیوژن مجدد: پیچ خوردگی به مدت ۲ ساعت و اصلاح پیچ خوردگی، نانوذرات کورکومین ۱۰: دریافت ۱۰ میلی‌گرم/کیلوگرم نانوذرات کورکومین به صورت داخل صفاقی ۳۰ دقیقه پیش از جراحی و سپس پیچ خوردگی و اصلاح پیچ خوردگی بیضه باقیمانده، نانوذرات کورکومین ۲۰: دریافت ۲۰ میلی‌گرم/کیلوگرم نانوذرات کورکومین به صورت داخل صفاقی ۳۰ دقیقه پیش از جراحی و سپس پیچ خوردگی و اصلاح پیچ خوردگی بیضه باقیمانده. برداشت یک‌طرفه بیضه سمت چپ یا راست بلافاصله در روزهای ۰ و ۱۲ انجام گرفت. بعضی از بیضه‌های پیچ‌خورده و اصلاح‌شده باقی گذاشته شدند و ۶۰ روز پس از جراحی نمونه‌گیری انجام شد. از یک گروه اضافی (۵ تایی) بدون هیچ‌گونه عمل جراحی به‌عنوان گروه کنترل نمونه‌برداری شد. نمونه‌های همونیزه بافت بیضه در روزهای ۱، ۱۲ و ۶۰ گرفته شدند و عصاره‌های آبی آن‌ها جمع‌آوری شده و آنزیم‌های سوپر اکسید دیسموتاز، مالون دی‌آلدئید و گلوتاتیون پراکسیداز ارزیابی شدند. اثر تیمار و روز نمونه‌گیری بر روی متغیرها با استفاده از روش آنالیز واریانس دوطرفه تجزیه و تحلیل شد.

**نتایج -** نتایج مطالعه تعادل بهتری در مقادیر گلوتاتیون پراکسیداز، سوپر اکسید دیسموتاز و مالون دی‌آلدئید را به دنبال پیچ خوردگی بیضه نشان داد. حیوانات درمان شده با نانوذرات کورکومین (در هر دو دوز تجویز شده) مقادیر بیشتری از آنزیم‌ها را نسبت به گروه‌های درمان‌نشده نشان دادند ( $p < 0.001$ ).

**نتیجه‌گیری و کاربرد بالینی -** در کل می‌توان چنین بیان کرد که تزریق داخل صفاقی نانوذرات کورکومین ممکن است در به حداقل رساندن آنزیم‌های مرتبط با استرس اکسیداتیو پس از ایسکمی/پرفیوژن مجدد بیضه در موش صحرایی کمک کند.

**واژه‌های کلیدی:** نانوذرات کورکومین، آنتی‌اکسیدان، بیضه، موش صحرایی