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Protective effect of curcumin on priapism and ischemia-reperfusion injury in rats

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Abstract. – OBJECTIVE: We aimed to identify the oxidative stress effects of the ischemic priapism on cavernosal tissues and to assess the biochemical and histopathological effects of curcumin in rats.

MATERIALS AND METHODS: 26 adult male Sprague Dawley rats were randomly divided into three groups. Group 1 (Control, n = 8): only penectomy was performed and 3 ml blood samples were obtained from the vena cava inferior (VCI). Group 2 (ischemia-reperfusion group; n= 8): penectomy was performed after 1 hour ischemic priapism + 30 min reperfusion and 3 ml blood samples were obtained from the VCI. Group III (IR + CURC group, n = 10): 200 mg/kg/day curcumin per orally before surgery for 7 days + penectomy after 1 hour ischemic priapism + 30 min reperfusion and 3 ml blood samples from the VCI. Total oxidant status (TAS), total antioxidant status (TAS) and paraoxonase (PON1) levels were measured. Tissue samples were investigated and scored histopathologically in terms of bleeding, edema and necrosis.

RESULTS: TOS levels were higher (p = 0.002), and TAS levels were lower (p = 0.001) in the IR group compared to the control group. As a result of curcumin treatment, TAS levels were increased (p = 0.003), and TOS levels were decreased (p = 0.004) in the IR + CURC group compared to the IR group. In the treatment group (IR + CURC) TAS and TOS levels were similar to levels in the control group. PON1 levels were increased with ischemia-reperfusion (p = 0.21) and decreased with curcumin treatment (p = 0.53), however these changes were not statistically significant. There was no significant difference in the effects of curcumin on histopathological changes.

CONCLUSIONS: This study showed that curcumin has preventive effects on oxidative stress parameters against ischemia-reperfusion injury.

Key Words:
Priapism, Oxidative stress, Paraoxonase, Curcumin, Rats.

Introduction

Priapism originated from the Greek word "Priapus" and is defined as a prolonged erection independent from sexual desire, orgasm and arousal[1]. Priapism may be classified as high flow, low flow and recurrent priapism. Low flow (ischemic) priapism is the most frequent type with a prevalence of 95%. Ischemic priapism which lasts longer than four hours is a compartment syndrome which requires urgent intervention. It leads to hypoxia, acidosis and fibrosis resulting in erectile dysfunction[2].

Reperfusion results from detumescence in the ischemic erectile tissue. Although reperfusion is required for repair mechanisms of the ischemic tissue, reperfusion injury is induced by production of an ample amount of free oxygen radicals through the restoration of oxygen supply and blood flow. Free oxygen radicals (FOR) lead to oxidative injury of various macromolecules like lipid, protein, carbohydrate, nucleic acid due to unshared electrons[3,4]. Oxidative injury develops with increased FOR and free radicals' exceeding the natural anti-oxidant mechanisms.

Curcumin was shown to reduce oxidative stress and tissue injury in IR injury in kidneys, heart, brain tissues and liver through its anti-oxidant properties[5], while it was reported otherwise[6]. This experimental study was conducted with the aim of defining the oxidative effect of priapism in cavernosal tissue in rats and to evaluate the anti-oxidant effect of curcumin on biochemical parameters and histopathological changes. This is the first experimental study in literature which investigates the effects of curcumin in the experimental rat priapism model.

Materials and Methods

This experimental study was conducted in the laboratories of Dicle University Health Sciences

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Research and Training Center between June-July 2012 after being granted approval by the Ethics Committee (date: 05.06.2012, number: 2012-30).

A total of 26 male adult Sprague-Dawley rats weighing between 280-320 g were used in the study. Subjects were selected and two groups of 8 and one group with 10 rats were formed. The rats were randomly allocated to Control, Ischemia-Reperfusion (IR) and curcumin + Ischemia-Reperfusion (CURC + IR) groups. All rats used in the study were kept in the same laboratory environment during the week prior to the procedure and fed with standard laboratory feed and water. 200 mg/kg of curcumin was given to the rats in CURC + IR group with gastric lavage.

Anesthesia was provided with 5-10 mg/kg of xylazin hydrochloride (Rompun®, Bayer-Istanbul) and 50-70 mg/kg of ketamine hydrochloride (Ketalar®, Pfizer-Istanbul) administered via intramuscular route. The operations were performed in an environment which was not sterile but appropriate for local asepsis-antisepsis conditions. The rats were placed on the operating table in the supine position post-anesthesia. The edge of 5 cc syringe was cut as appropriate for the flaccid penile root and a mechanism was constituted to apply vacuum to penis. A 16 Fr Foley catheter was cut in 2 mm length as constriction band and placed in penile root suitable for erection vacuum mechanism.

Group I. Control group (n:8): Only penectomy was performed and 3 ml of blood was obtained from VCI.

Group II. Ischemia reperfusion group (IR) (n:8): 60 min of ischemic priapism was constituted. Afterwards the band was removed from penile root and reperfusion of penile tissue was allowed during 30 min. Penectomy was done to evaluate the effects of ischemia/reperfusion and 3 ml of blood samples were obtained from VCI for assessment of biochemical parameters for the study.

Group III. Study group (CURC + IR) (n:10): A single dose of 200 mg/kg curcumin was administered in tap water as gastric lavage using a No 6 silastic orogastric feeding tube during the 7 days prior to the procedure. Afterwards, 60 min priapism was constituted. Penectomy was performed after 30 min of reperfusion had been provided and blood was obtained from the VCI. The removed penile tissue was put into 10% form aldehyde for histopathological examination. The blood samples were centrifuged at 4000 rpm for 10 min and the obtained plasma was transferred into plastic Eppendorf tubes for biochemical analysis and stored at –20°C.

Total Anti-Oxidant Status (TAS) Measurement

Total anti-oxidant capacity was measured with the Erel method[6]. 2,2´-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS radical) is used in this method. ABTS radical loses its blue or green color depending on anti-oxidant concentration and anti-oxidant capacity. This color change is evaluated by measuring absorbance value at 660 nm. The principle of this method is based on ABTS molecule's oxidizing to ABTS+ molecule in presence of hydrogen peroxide. The radical which is dark green in 30 mmol/L acetate buffer and pH 3.6 becomes light green in 0.4 mmol/L acetate buffer and pH 5.8. There is an inverse relationship between color change and the anti-oxidant amount in the sample. Reaction velocity is calibrated with a standard method, Trolox (Trolox equivalnet/L)[6].

Preparation of Reactives

Reactive 1: 0.4 mol/L of acetate buffer (pH:5.8) was formed by melting 32.8 g CH3COONa within 1000 ml distilled water. 22.8 ml acetic acid was diluted with 1000 ml water and made 0.4 mol/L in concentration. 940 ml sodium acetate solution and 60 ml acetic acid solution were mixed.

Reactive 2: 30 mmol/L of acetate buffer (pH:3.6) was formed by melting 2.46 g CH3COONa within 1000 ml distilled water. 1.705 ml acetic acid was diluted with 1000 ml distilled water and a mixture in 30 mmol/L in concentration was obtained. 75 ml sodium acetate solution and 925 ml acetic acid solution were mixed (pH:3.6). Afterwards 278 µl H_2O_2 solution was diluted with 1000 ml buffer solution and concentration was made as 2 mmol/L. Then 0.549 g ABTS radical was melted in 100 ml solution and concentration was made 10 mmol/L, waited at room temperature for one hour and characteristic ABTS color was provided to obtain.

Aeroset was applied to automatic analyzer (Abott Aeroset® C8000™ device) after spectrophotometric adjustments had been done.

Total Oxidant Status (TOS) Measurement

Measurement was done with a full automatized colorimetric method developed by Erel[6].

Preparation of Reactives

Reactive 1: The main solution was prepared by dissolving 140 mM NaCl solution with 25 mM H_2SO_4. First 10% glycerole was dissolved in the main solution and totally 250 μM Xylenol orange was dissolved and the solution prepared.

Reactive 2: First 10 mM o-Dianisidine dihidrocloride was dissolved within the main solution and then 5 mM ammonium ferrous sulphate was dissolved and the reactive was prepared. The oxidants in the sample oxidizes ferrous ion-o-dianisidine complex to ferric ion. Glycerole in the environment facilitates this reaction and makes the velocity three fold. Ferric ions form a color complex with xylenol orange in acidic environment. The degree of the color which is proportional with the amount of oxidants is measured spectrophotometrically (μmol H_2O_2 Eqv./L).

Paraoxonase (PON 1 Measurement)

Serum PON1 levels were spectrophotometrically measured with the modified Eckerson method[7]. Initial ratios of paraoxone (0.0-diethyl-0-p-nitrophenylphosphate; Sigma Chemical Co., London, UK) hydrolysis was determined with measurement of free p-nitrophenol at 37°C in 412 nm wave length.

Reactive 1: 0.157 g Tris (2,3-diethyl-4-nitrophenyl) was dissolved in some distilled water, 60 micron Tris (2,3-diethyl-4-nitro-phenyl) phosphate was added and completed to 10 ml with distilled water and pH was made 7.4.

Reactive 2: 0.0825 g $CaCl_2$ was completed to 250 ml with distilled water. The reactives for PON1 test were prepared using the chemicals in our laboratory and stored at 4-8 °C.

Histopathological Examination Method

Histopathological examination of the tissues were done at Department of Pathology, Dicle University. After the tissues had been fixed in 10% form aldehyde for 24 hours, they were removed and routine histopathological follow up was completed. They were, then, embedded into paraffine blocks, 4 μm of sections were taken, stained with Haematoxylin-Eosin (H&E) stain and examined under light microscopy (Nikon ECLIPSE 80i, Tokyo, Japan) by an expert pathologist. Subcutaneous tissue, corpus cavernosum, corpus spongiosum, urethra, vascular endothelium, hemorrhage and necrobiosis-necrosis changes were evaluated separately and reported as no change (0), mild (1), moderate (2) and severe (3). Epithelial-subepithelial hemorrhage and edema in the IR group is shown in Figure 1. CURC+IR groups' subepithelial vascular congestion and edema with focal signs of bleeding and necrobiotic changes is shown in Figure 2. The histopathological examination results of the study groups and the control group were analysed with statistical analyses.

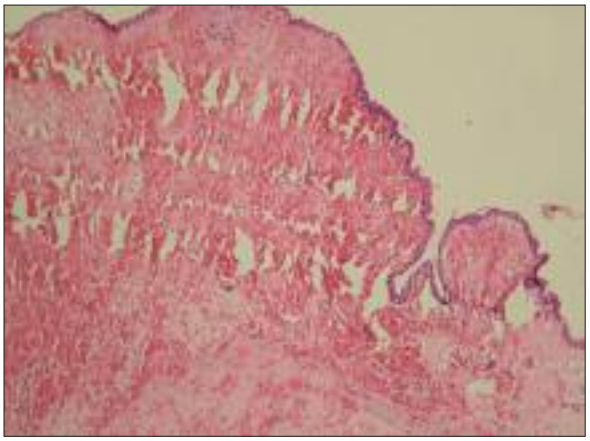

Figure 1. IR groups. Epithelial-subepithelial hemorrhage and edema. (H&E, ×100).

Statistical Analysis

A SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA) 18.0 program was used for statistical analysis. Data were presented as mean ± standard deviation. The single sample Kolmogorov-Smirnov test was applied for determining whether the data were nor-

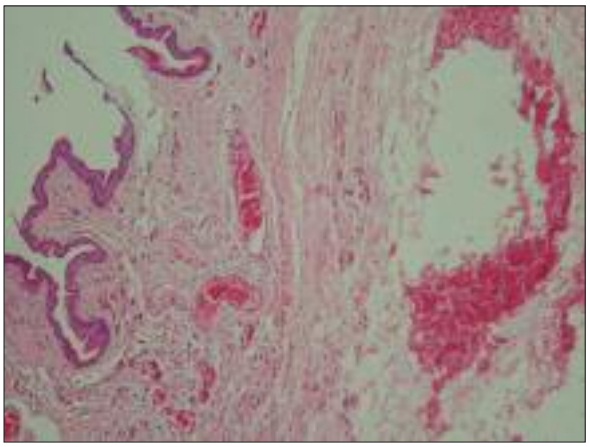

Figure 2. CURC+ subepithelial vascular congestion and edema with focal signs of bleeding and necrobiotic changes. (H&E, ×100).

mally distributed. The Chi-square test, Kruskal-Wallis test and Mann-Whitney U test were applied for determining inter-group differences. A p level of < 0.05 was accepted as statistically significant.

Results

Results of Biochemical Analysis

When the TAS values of the three groups were compared, while a statistically significant difference was not seen between the CURC + IR group and control group ($p = 0.28$), there was a significant difference between the IR group and control group ($p = 0.001$). Similarly, the difference between the IR + CURC group and IR group was also statistically significant ($p=0.003$). The reduction in TAS in the IR group was seen to approximate to that in the control group after curcumin treatment.

When TOS values of the three groups were compared, while a statistically significant difference was not seen between the CURC + IR group and control group ($p = 0.98$), there was a significant difference between the IR group and control group ($p = 0.002$). Similarly, the difference between the IR + CURC group and IR group was also statistically significant ($p = 0.004$). The increase in TOS in the IR group was seen to approximate to that in the control group after curcumin treatment.

The three groups were compared with regard to paraoxonase (PON1) and no difference was observed. p value was found as 0.21 between the control and IR group, 0.53 between the IR group and CURC + IR group and 0.18 between the control and CURC + IR group. We observed that IR application decreased PON1 values, PON1 level increased with curcumin and approximated to the levels of the control group, however these changes did not reflect statistical measurements. TAS, TOS, and PON1 values of the groups are shown in Table I.

Comparison of Hstopathological Examination Results

Histopathological examination results are shown in Table II. Histopathological examination was done separately for hemorrhage, edema, necrosis and they were scored. The scores of histopathological examination results are shown in Table III.

While no change was observed with regard to hemorrhage and necrosis in all tissue samples in the control group, mild edema was observed in only 2 tissue samples. In the IR group, there was mild hemorrhage in 25% of the samples, moderate hemorrhage in 50% and severe hemorrhage in 25% ($p = 0.148$). In this group, moderate edema was observed in 50% of the samples and severe edema was observed in 50% ($p = 0.318$). While no change was observed in 25% of the samples in the same group, there was mild necrosis in 37.5% and moderate necrosis in 37.5% ($p = 0.060$). Mild hemorrhage was observed in 60% of the samples and moderate hemorrhage was observed in 40% of the samples in CURC + IR group ($p = 0.00$). In this group, moderate edema was seen in 50% of the samples and severe edema was seen in 50% of the samples in the same group ($p = 0.002$). While no change was observed in 70% of the samples in the same group, mild necrosis was seen in 30% ($p = 0.090$).

When the groups were compared with regard to histopathological scoring, hemorrhage in the IR group was seen to be significantly higher compared to the control group ($p = 0.001$). Although hemorrhage in the IR group was seen to decrease after curcumin treatment, this difference was not statistically significant ($p = 0.148$).

When groups were compared with regard to histopathological scoring, edema was seen to be statistically significantly higher in the IR group compared to the control group ($p=0.001$). Although the mean edema score in the IR group was seen to decrease after curcumin treatment, this decrease was seen not to be statistically significant ($p = 0.318$).

Table I. TAS, TOS, PON1 values of the groups and mean ± SD values.

	Group		
	Control (n = 8)	IR (n = 8)	CURC + IR (n = 10)
PON1 mean ± SD U/ml	141.64 ± 30.32	112.05 ± 29.57	128.15 ± 35.45
TOS mean ± SD μmol H_2O_2 Eqv./L	34.42 ± 10.11	67.38 ± 15.00	38.14 ± 17.70
TAS mean ± SD (TroloxEq/L)	0.19 ± 0.04	0.06 ± 0.04	0.14 ± 0.08

Table II. Histopathological examination results.

		Histopathological changes									
		No (0); mild (1); moderate (2); severe (3)									
Group		1	2	3	4	5	6	7	8	9	10
Control	Hemorrhage	0	0	0	0	0	0	0	0		
	Edema	0	1	0	0	1	0	0	0		
	Necrosis	0	0	0	0	0	0	0	0		
I/R	Hemorrhage	3	2	3	2	1	2	1	2		
	Edema	3	3	2	2	2	3	2	3		
	Necrosis	2	2	2	1	0	1	0	1		
CURC + I/R	Hemorrhage	1	1	1	2	2	1	1	2	2	1
	Edema	2	2	2	3	2	2	1	2	3	2
	Necrosis	0	0	0	1	1	0	0	1	0	0

When the groups were compared with regard to histopathological scoring, necrosis in the IR group was seen to be significantly higher compared to the control group ($p = 0.008$). Although the mean necrosis seen in the IR group was seen to decrease after curcumin treatment, this decrease was seen to be statistically insignificant ($p = 0.060$).

Discussions

Priapism is defined as prolonged erection emerging with impairment of the mechanisms managing penile tumescence, rigidity and flaccidity[1]. Ischemic priapism is the most common type (95%). It is a condition which develops due to insufficient detumescence mechanism and may lead to permanent injury in erectile tissues.

Treatment provides blood flow to the ischemic tissue. However paradoxically, reperfusion of the ischemic tissue leads to much severe tissue injury depending on the severity of ischemic injury[8]. Many mechanisms play a role in the injury seen during the reperfusion period, mainly rapidly developing free oxygen radical (FOR) species with molecular oxygen entrance into the cell. The most susceptible cellular structures to reperfusion injury are membrane lipids, proteins, nucleic acids and deoxyribonucleic acid molecules[9]. FOR formation leads to lipid peroxidation, polysaccaride depolymerization and destruction of DNA structure. Metabolic and structural changes develop in the cell in ischemic period. Cellular oxidative phosphorylation decreases with disruption of blood flow to the tissue and high energy phosphate synthesis like adenosine 5'-triphosphate and phosphocreatine decreases[10]. The cell membrane Na^+, K^+-ATPase pump is inhibited when energy storages of the cell are emptied. Consequently intracellular Na^+ and Ca^{2+} ion concentrations increase[11]. Intracellular Ca^{2+} increase is cytotoxic for the cell[12]. Leukocyte adhesion molecules of proinflammatory cytokines increase and

Table III. The scores of histopathological examination results.

Group		Avg. Score	Histopathological scores (%)				
			No (0)	Mild (1)	Moderate (2)	Severe (3)	p
Control	Hemorrhage (n = 8)	0	100.0%	.0%	.0%	.0%	0.001*
Control	Edema (n = 8)	0.25	75.0%	25.0%	.0%	.0%	0.001*
Control	Necrosis (n = 8)	0	100.0%	.0%	.0%	.0%	0.008*
IR	Hemorrhage (n = 8)	2	.0%	25.0%	50.0%	25.0%	0.148**
IR	Edema (n = 8)	2.5	.0%	.0%	50.0%	50.0%	0.318**
IR	Necrosis (n = 8)	1.12	25.0%	37.5%	37.5%	.0%	0.060**
CURC + IR	Hemorrhage (n = 10)	1.4	.0%	60.0%	40.0%	.0%	0.000***
CURC + IR	Edema (n = 10)	2.1	.0%	10.0%	70.0%	20.0%	0.002***
CURC + IR	Necrosis (n = 10)	0.3	70.0%	30.0%	.0%	.0%	0.090***

*Control group with IR **IR group with CURC + IR ***control group with CURC + IR.

anti-oxidant enzyme formation decreases with the changing cellular ion concentration during this period. This condition makes the cell susceptible injury during the reperfusion period.

The pro-oxidant/anti-oxidant balance of the organism is of great importance for maintaining health. Endogenous and exogenous anti-oxidants form the anti-oxidant defense mechanism. The anti-oxidant defense system protects the organism by preventing FOR formation and related injuries and providing detoxification. Harmful effects of free radicals are reduced or completely eliminated by some substances which show exogenous anti-oxidant property. Many substances with proposed anti-oxidant properties were evaluated by testing in various IR models. Curcumin is an anti-oxidant agent of which anti-oxidant, anti-inflammatory, immunomodulatory, antitumoral and antipsoriatic effectiveness has been proven in many studies[13]. It affects the cyclooxygenase, glutathione-s-transferase, immunemodulatory effect, angiogenesis and cell-cell adhesion effect beside anti-oxidant effect in acidic and neutral pH. It shows its anti-oxidant effect through deactivating reactive oxygen metabolites and reactive nitrogen metabolites[14,15]. It also shows indirect anti-oxidant effects through increasing glutathione synthesis[16]. Curcumin was selected as the anti-oxidant agent in our study due to these reasons.

In the studies about ischemia reperfusion in cavernous tissues, 60 min was shown to be sufficient for creating IR injury in cavernous tissues[17]. 60 min and 30 min of reperfusion was applied in this experimental priapism model, consistent with the literature. In literature, two different models were described to develop priapism in an animal model. Priapism may be developed by using a pharmacologic agent (papaverin, PgE1) or a constriction band placed in the penile root[18,19]. In our study, priapism was created by applying a constriction band which has advantages like being inexpensive, easily applicable in little animals like rats and maintaining priapism during a desired period.

There are a limited number of studies investigating ischemia reperfusion injury in erectile tissues. However there is an ample amount of studies investigating IR injury in other organs[6,20]. TAS and TOS which objectively show tissue injury and treatment level were studied due to being more practical and inexpensive. Our study is the first study in literature which investigates TAS, TOS in the IR model in erectile tissues.

Curcumin was shown to reduce both oxidative stress and lipid peroxidation in the studies done on different tissues[21-23]. Although the studied tissues were different, the TOS levels in our study were also observed to significantly increase and the TAS levels were significantly decreased in the IR group. However TAS and TOS levels were detected to approximate to the control group in curcumin group and it was consistent with the literature, may be effective for prevention of IR injury that may develop following priapism treatment.

Paraoxonase (PON1) is an anti-oxidant enzyme known to hydrolyse lipid peroxides. PON1 hinders LDL oxidation. In a study[24], increased PON1 level was reported to be related with increased anti-oxidant level. In another study[25], PON1 levels were found high in the IR group as a protective effect. In our study, PON1 level statistically significantly decreased in the IR group compared to the control group and increased in the CURC + IR group. Curcumin is considered to show an anti-oxidant effect by increasing PON1 level although it did not lead to a statistically significant increase.

In experimental studies[26,27] conducted with rats, various scoring and function parameters were reported for evaluating IR injury at tissue level depending on the histological structure and functions of the related tissue. In our study, tissue ischemia, necrosis and edema were scored separately in order to histologically evaluate the effectiveness of curcumin. While positive changes were detected in ischemia, necrosis and edema scores in the curcumin group, this increase was not found statistically significant.

Conclusions

This is the first study which shows the effectiveness of curcumin in the priapism model and evaluates TAS, TOS, PON1 and histological findings. In our study, it was shown that IR injury develops in priapism and this injury may be prevented with curcumin. We consider that our study will shed light on further studies on this issue. However, randomized controlled studies are required to clinically show the effectiveness of curcumin.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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