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Barbaloin Attenuates Oxidative Testicular Injury Induced by Ischemia Reperfusion via Antioxidant Effects

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Abstract. The purpose of this research is to examine the protective effects of barbaloin on testes injury induced by ischemia reperfusion. In the experimental stage of our research, the rats were assigned to 4 groups. Our groups are scheduled as follows; sham, ischemia reperfusion, ischemia and reperfusion + DMSO and ischemia reperfusion+barbaloin. Some oxidant and antioxidant parameters were evaluated in testes tissues obtained at the end of the experiment. In ischemia reperfusion group it was found out that the oxidant parameters increased and antioxidant parameters decreased but on the contrary, the antioxidant parameters increased and oxidant parameters decreased in the treatment group. These results demonstrated that barbaloin administration performed a protective effect against oxidative testes injury induced by ischemia reperfusion.

1. Introduction

Among adolescents and infants, testes torsion is a critical urological emergency and may cause testicular necrosis and complete damage. At the early stages, diagnosis and treatment are important points to restrain infertility because of its 1 in 158 incidence by 25 years old age in males [1, 2]. As an urological emergency health condition, testes torsion often takes place in adolescents and children. A seasonable and effective cure must be applied to able to maintain testicular function. Detorsion of twisted testes is a part of the treatment. Testicular torsion detorsion (T/D) pathophysiology is described as acute ischemia reperfusion (I/R) injury [3, 4]. When the reactive oxygen species (ROS) are produced excessively, this may be a major reason of inducing I/R injury during testicular T/D [5, 6]. Spermatic cord T/D, a testicular I/R state, leads to testicular injury [7]. If it is untreated, testicular torsion may reduce fertility [8]. In rat spermatic cord torsion model, permanent spermatogenesis cessation occured due to germ cell spesific oxidative stress, inflammation and apoptosis [9]. Recent studies about oxidative stress and free oxygen radicals arising from testicular torsion demonstrated that antioxidants prevents ROS- induced testes injury [8, 10–12]. As carrying high economic value, Aloe is a *A Liliaceae* family member plant [13, 14]. Aloe plays role in immunity

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and acts via anti-oxidation and anti-inflammatory effects [13], and widely preferred in clinic [15]. Barbaloin, main ingredient in aloe, catches on from day to day [16]. Barbaloin (10-b-D-glycopyranosyl-1,8-dihydroxy-3-hydroxymethyl-9 (10H) -anthracenor) is the main compound of Traditional Chinese medicine aloe vera from the plant liliaceous. According to previous studies, many pharmacological effects of barbaloin have been shown, including anti-oxidant, anti-inflammatory and anti-tumor effects [17]. Different agents with anti-inflammatory, antioxidant and radical scavenging features, and it has been reported that they have beneficial effects in alleviation or elimination of I/R injuries [18–20]. This study was planned to search the protective effect of barbaloin against testicular oxidative damage induced by I/R.

2. Methods

2.1. Animals and Ethical Approval

The present study was admitted by Atatürk University Experimental Animal Ethics Committee (2019-66). Experiments of this study were performed at Atatürk University Experimental Animals Research and Application Center (ATADEM). Wistar albino male rats weighing 270-300 g, acquired from Atatürk University Experimental Animal Research and Application Center. Rats were housed in cages in laboratory conditions such as temperature of 22 ± 2 $^{\circ}C$, humidity of 55 ± 5% , and 12 light/12 darkness. Rats were fed with standard rat feed, and supplied drinking water. All animals were food deprived for 12 hours before the experiment, but were allowed to drink water.

2.2. Groups and Experimental Design

32 male rats were allocated into 4 experimental groups (n=8): Sham group; only laparotomy was made under anaesthesia. Ischemia reperfusion (I/R) group; 2 hours ischemia and 2 hours reperfusion was performed. In the midline field of lower abdomen, a longitudinal and 2 cm length incision was applied. After a small peritoneal incision, spermatic cords and testes were located. In I/R group, bilateral testes ischemia was carried out via a vascular clamp by applying below the testes. Ischemia reperfusion+DMSO (I/R+DMSO) group; 2 hours ischemia was fullfilled and DMSO 0.3 ml administered as intraperitoneal (i.p.). Then, 2 hours reperfusion stage was done. Ischemia reperfusion+barbaloin (I/R+barbaloin) group; 2 hours ischemia was done. Following, 20 mg/kg i.p. application of barbaloin (Sigma-Aldrich Co, USA) and then 2 hours reperfusion was carried out. All experimental steps were performed under deep anaesthesia by administration of ketamine-xylazine 60/10 mg/kg i.p. (Ketalar 50 mg/ml Pfizer İlaçları Limited Şirketi, İstanbul and Xylazine Rompun BAYER İstanbul, Turkey). The testes were cleaned in cold saline and then stored in a freezer at $-80^{\circ}C$ for biochemical measurements.

2.3. Biochemical Assessments

Total antioxidant status (TAS) value was detected with the commercial kit (Rel Assay Diagnostics). Total oxidant status (TOS) measurement was applied with commercially available kit (Rel Assay Diagnostics). TOS to TAS ratio was admitted as the oxidative stress index (OSI). OSI value was measured as follows: OSI = [(TOS, μPmol H2O2 equivalent L)/(TAS, mmol Trolox equivalent/L) $\times$ 10]. We preferred OSI as another indicant of oxidative stress. Xanthine and xanthine oxidase system produce superoxide radicals. Superoxide radicals, react with nitroblue tetrazolium to form formazan dye, form a basis of superoxide dismutase (SOD) evaluation [21]. Amounts of lipid peroxidation in testes tissue were measured by assessing malondialdehyde (MDA) using the thiobarbituric acid test [22]. The activity of myeloperoxidase (MPO) in the testes tissue was estimated according to methods described by Bradley et al. [23].

2.4. Statistical Analysis

Results were analyzed using One-way ANOVA and then Tukey test for pairwise comparisons of groups. All the results were presented as Means ± SEM. The differences were accepted significant when $p < 0.05$.

3. Results

3.1. Biochemical Results

In this study, when SOD, MPO activities and MDA levels were analyzed, it was found that MPO activity and MDA levels were significantly increased in I/R group, whereas SOD enzyme activity decreased due to insufficient antioxidant capacity. In addition, it was determined that SOD activity was increased by supporting antioxidant capacity in the group treated with barbaloin compared to I/R group but MPO activity and MDA levels decreased (See Table 1). When the findings of OSI, TAS and TOS levels were evaluated, it was found that TAS levels significantly decreased while TOS and OSI levels significantly increased in I/R group compared to sham and IR+DMSO groups ($P < 0.05$). However, TAS levels increased but TOS and OSI levels decreased in group treated with 20 mg/kg barbaloin (See Table 2).

Table 1: The Minimum, Maximum, Mean and Standard Error of Mean values of Superoxide Dismutase (SOD), Myeloperoxidase (MPO) activities of all experimental groups were presented.

Groups		MDA (µmol/gr tissue)	SOD (U/mg protein)	MPO (U/mg protein)
Sham	Minimum	164,83	342,52	28354,84
	Maximum	228,42	478,85	41325,94
	Mean	205,7816*	406,4826*	34973,9064*
	Std. Error of Mean	8,30084	15,44626	1401,19425
I/R	Minimum	359,29	163,54	76431,64
	Maximum	492,67	216,90	93245,80
	Mean	419,0717*,**	192,5102*,**	85023,8419*,**
	Std. Error of Mean	16,39499	5,96061	2102,09375
I/R+DMSO	Minimum	351,44	171,43	79304,19
	Maximum	503,22	223,33	93278,67
	Mean	423,4385*,#	194,0845*,#	85452,2270*,#
	Std. Error of Mean	20,61832	6,17502	1622,62726
I/R+ Barbaloin	Minimum	184,76	362,68	30426,12
	Maximum	221,44	447,45	40326,99
	Mean	208,8152**,#	408,3420**,#	35969,6675**,#
	Std. Error of Mean	4,88605	11,08708	1122,94026

*, **,# represent the statistically significant relationship between the groups. p value is less than 0.001.

Table 2: The Minimum, Maximum, Mean and Standard Error of Mean values of Total Antioxidant Status (TAS), Total Oxidant Status (TOS) and Oxidative Stress Index (OSI) levels of all experimental groups were presented.

Groups		TAS (mmol/L)	TOS (µmol/L)	OSI
Sham	Minimum	1,13	5,90	,43
	Maximum	1,62	7,78	,66
	Mean	1,3666*	6,9316*	,5141*
	Std. Error of Mean	,05620	,20719	,02918
I/R	Minimum	,63	9,78	1,12
	Maximum	,97	12,79	1,91
	Mean	,7842*,**	11,5362*,**	1,4955*,**
	Std. Error of Mean	,03817	,36801	,08720
I/R+DMSO	Minimum	,74	10,69	1,23
	Maximum	,90	13,56	1,67
	Mean	,8256*,#	11,8911*,#	1,4475*,#
	Std. Error of Mean	,02275	,33126	,05533
I/R+ Barbaloin	Minimum	,94	6,33	,47
	Maximum	1,59	7,65	,72
	Mean	1,3198**,#	7,2657**,#	,5613**,#
	Std. Error of Mean	,06976	,16209	,03165

*, **,# represent the statistically significant relationship between the groups. p value is less than 0.001.

4. Discussion

Testicular torsion is an urological health condition and may occur among adolescents, newborn males and children. A fast diagnosis and surgical interfedence matters against permenant fertility loss and testicular injury [24]. In order to prevent testicular injury, quick diagnosis and right management must be performed [25]. Testicular T/D leads to I/R injury, a harmful pathological situation. I/R injury causes to excessive free radicals production like ROS and takes role in inflammatory signaling pathway activation [26]. Free oxygen radicals, produced during I/R injury, can lead to oxidative damage in many cellular biomolecules containing proteins, lipids, and DNA [6, 27] By virtue of ischemia, oxygen supply reduces, cellular energy depletes and toxic metabolites accumulate and hereat, oxidative stess, germ cell death occur. ROS and reactive nitrogen species are much more produced due to reperfusion and these species lead to membrane lipid peroxidation. In conclusion tissue injury occurs, cell structure and function disorganize [28]. Reperfusion stage damages the tissue much more than the ischemic phase [29]. At the reperfusion period, ROS production is the key for uncontrolled oxidative stress and increased amounts of ROS may promote the inflammatory cascade [30]. Several studies have been reported that testicular I/R incremented the oxidative stress and decreased the level of "antioxidant enzymes" [31, 32]. Antioxidants take role in breaking down of chain oxidative reactions and reducing oxidative stress [33, 34]. Glutathione (GSH) and SOD are examples for the natural free radical scavengers which conserve tissues and organs injuries induced by ROS. ROS related injury and lipid peroxidation increase in case of a depletion in the amount of renal antioxidants (catalase, SOD, GSH and other free radical scavengers) [35]. For the male reproductive organs, glutathione poeroxidase and SOD are the main enzymes which scavenge harmful ROS [36]. TAS and TOS reflect the redox balance between oxidation and antioxidation. TAS measurement is an indicator of the activity of all antioxidants while TOS is an indicator of ROS [37, 38]. Oxidative stress is an oxidant-antioxidant imbalance status, due to oxidants which exceed the antioxidant capacity. OSI is the ratio of TOS to TAS and is an indicator of OSI degree [37, 38]. TAS and TOS are the well known methods in the biochemical analysis of I/R injury studies [39]. I/R study was showed that the TOS and OSI values increased, in contrast to the total antioxidant status decreased in the I/R group [40]. In a study, it was shown that SOD levels increased effectively with barbaloin [41]. Barbaloin alleviated lipopolisaccaride-induced acute lung injury by reducing intracellular ROS [42]. As the most deleterious free radical effect, lipid peroxidation in cells results in decrease in membrane potential and right after, cell injury. MDA occurs due to lipid peroxidation. It induces polymerization and causes cross linking in membrane components that result in serious cell damage [43]. MDA is preferred to evaluate free radical formation in postischemic tissue [44]. MDA represents lipid peroxidation degree and indirectly shows I/R injury degree [45]. MPO is particularly within neutrophils and so an increase in MPO levels also is an indicant for neutrophil activation [46]. Barbaloin-related MDA and MPO levels were not found in the literature, and in this study, it was significant that it was the first to reduce these levels. The most important biochemical data of barbaloin treatment can be expressed as follows: The I/R injury in testes was related to dramatic increases of MDA level, TOS and OSI values and MPO activity, and a decrease in SOD activity and TAS value in the testicular tissue. The novel result of the present study is that barbaloin significantly derogated testicular tissue damage induced by I/R. Barbaloin treatment effected in the positive direction changes of the findings of MDA, TOS, OSI and MPO and stimulated an overproduction of enzymatic antioxidant SOD activity and increased TAS value.

5. Conclusion

These results recommend that barbaloin may protect the testes by diminishing oxidative injury caused by I/R. We have found that treatment with barbaloin at single dose (20 mg/kg) reduces testicular damage induced by I/R in testes in experimental animals exposed to a torsion for 2 h and detorsion for 2 h model. Part of the mechanisms of these protective effects of barbaloin may be stemming from supporting the antioxidant capacity by barbaloin. New studies may be helpful to be able to find different protective mechanism on I/R-induced testicular tissue injury.

6. Conflict of interest

None

7. Financial Disclosure

There is no financial support organization in the implementation of this study.

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