

## Ternatin pretreatment attenuates testicular injury induced by torsion/detorsion in Wistar rats<sup>1</sup>

Pré-tratamento com ternatina atenua a lesão testicular induzida por torção/destorção em ratos Wistar<sup>1</sup>

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### ABSTRACT

**Purpose:** To investigate the possible protective role of the bioflavonoid ternatin (TTN) when administered before induction of ischemia/reperfusion injury in rat testis. **Methods:** Thirty-six Wistar rats were randomly assigned to 3 groups (n=12), divided in 2 subgroups (n=6). Saline 2.0ml (G-1), dimethylsulfoxide (DMSO) 3% solution (G-2) or TTN 12 mg/kg/dose (G-3) was administered ip. to all rats, respectively, 21, 12 and 1 hour before torsion. Anesthetized rats were subjected to ischemia (3 hours) induced by 720° torsion of the spermatic cord. Right testis and arterial blood samples were collected at the end of ischemia (T-0), and 3 hours later (T-3) for assessment of testis malonaldehyde (MDA), reduced glutathione (GSH), and plasma total antioxidant power (TAP). **Results:** MDA decreased significantly (p<0,001) in G-2 and G-3 in T-0 and T-3 timepoints. Additional decrease in MDA was seen in G-3 after 3 hours of reperfusion (T-3). GSH increased significantly in G-2 (p<0.001) and G-3 (p<0.05) at the end the ischemia (T-0). A significant increase in GSH was seen 3 hours after testis detorsion (T-3) in G-2 rats. TAP values remained unchanged. **Conclusion:** The data provides *in vivo* evidence of the antiperoxidative and antioxidative properties of TTN in tormented rat testis.

**Key words:** Flavonoids. Spermatic Cord Torsion. Ischemia. Reperfusion. Rats.

### RESUMO

**Objetivo:** Investigar o possível efeito protetor do bioflavonóide ternatina (TTN) quando administrado antes da indução da lesão de isquemia/reperfusão testicular em ratos. **Métodos:** Trinta e seis ratos Wistar, aleatoriamente distribuídos em três grupos (n=12) divididos em dois subgrupos (n=6) cada foram tratados com solução salina (G-1), dimetilsulfóxido (DMSO) 3% (G-2) ou TTN 12 mg/kg/dose (G-3), administrados i.p. 21, 12 e 1 hora antes da torção. Ratos anestesiados foram submetidos à isquemia (3 horas) induzida por torção (720°) do cordão espermático direito. Amostras (testículo ipsilateral e 3,0 ml de sangue arterial) foram coletadas ao final da isquemia (T-0), e 3 horas depois (T-3) para a avaliação das concentrações de malonaldeído (MDA), glutatona reduzida (GSH) no testículo e capacidade antioxidante total (TAP) no plasma. **Resultados:** MDA diminuiu significativamente nos grupos G-2 e G-3 nos tempos T-0 e T-3. Houve diminuição adicional no G-3 após 3 horas. GSH aumentou significativamente nos grupos G-2 (p<0,001) e G-3 (p<0,05) no T-0 e T-3 no G-2. TAP permaneceu inalterada. **Conclusão:** Os achados fornecem evidências *in vivo* das propriedades antioxidantes e antiperoxidativas da TTN na T/D do testículo do rato.

**Descritores:** Flavonóides. Torção do Cordão Espermático. Isquemia. Reperusão. Ratos.

## Introduction

Flavonoids are claimed to have protective effects against free radicals induced lipid peroxidation of living cell membranes<sup>1,2</sup>. Their protective effects may be due to their ability to scavenge reactive oxygen species<sup>3</sup>, free hydroxyl and peroxy radicals<sup>4</sup>, or to chelate iron ions<sup>5</sup>, which are involved in the initiation of free radical reactions. Ternatin (TTN), a tetramoxyflavone from *Egletes viscosa* L., is known to have protective effects against hepatic oxidative damage induced by aflatoxin B1, a metabolite of *Aspergillus flavus* and a potent hepatotoxic<sup>6</sup>.

Testicular torsion is a common urologic emergency among newborns, children and adolescents. Events occurring during testicular torsion/detorsion (T/D) are representative of an ischemia/reperfusion (I/R) type of injury observed in other organs<sup>7</sup>. Experimental unilateral spermatic cord torsion has shown to induce testicular biochemical changes indicative of lipid peroxidation<sup>8-11</sup>.

To our knowledge, no study has investigated the role of TTN in testicular I/R. In the present study, we evaluated the effect of TTN on testicular oxidative stress in a rat I/R injury model.

## Methods

Approval for experimental use of laboratory animals was obtained from the Committee of Ethics in Animal Research of the Federal University of Ceara (Protocol 14/02). Study groups consisted of 36 young Wistar male rats (*Rattus norvegicus albinus*, *Rodentia*, *Mammalia*), aged 55-65 days, weighing 175±14g, provided by the Faculty of Medicine Small Animals Breeding Facility (Federal University of Ceara). Rats were housed in polypropylene cages at ambient temperature of 24° on a 12 h light-dark cycle and allowed free access to food (Purina chow) and water until 12 h before the experiments.

Animals were randomly assigned to 3 groups (n=12): G-1 (Saline-control) G-2 (Vehicle) and G-3 (TTN). Rats of each group were distributed in 2 subgroups, each comprising 6 animals. Anesthetized (ether) rats were subjected to testis ischemia (3 hours) induced by 720° torsion of the spermatic cord. After each surgical intervention the incisions were closed and the rats were returned to their cages and allowed free access to water. At the end of the experiment all animals were killed by an overdose of anesthetics. Tissue samples were snap-frozen in liquid nitrogen and stored in glass tubes at -70° until subsequent preparation and analysis of testis homogenate. Plasma samples obtained from blood after 10 minutes of refrigerated centrifugation (4000 rotations/min) and

were likewise stored.

### Study groups

Group 1 (saline): saline 2.0 ml i.p., 21, 9 and 1 hour before ischemia.

Group 2 (vehicle): dimethylsulfoxide (DMSO) 3% in 0.9% saline, 10 ml/kg/dose completing the volume for 2.0 ml with saline, i.p., 21, 9 and 1 hour before ischemia.

Group 3 (TTN): TTN (1.2 mg/ml) i.p., suspended in DMSO 3% aqueous solution, 10 ml/kg/dose completing the volume for 2.0 ml with saline, 21, 9 and 1 hour before ischemia.

### Planta material

*Egletes viscosa* flower heads were collected during the dry season in northeast Brazil. Botanical identification was confirmed by Prof. A. G. Fernandes, of the Federal University of Ceará (UFC). The voucher specimen (#16327) is deposited in the *Herbarium Prisco Viana* (UFC). Ternatin was isolated from dried flower buds of *E. viscosa* L. as per procedures and methods described previously<sup>12</sup>.

### Chemicals

DMSO (purity grade: 99.9%) was purchased from Cromato Produtos Químicos Ltda. Total Antioxidant Power kit (TA-01) was purchased from Oxford Biomedical Research. All other chemicals were purchased from standard commercial sources and were of the highest quality available.

### Statistical methods

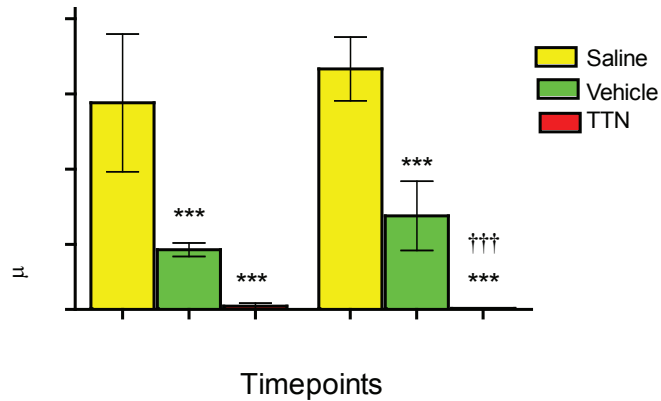
Graphpad Prism 5.0 (www.graphpad.com) was used for statistical analysis and graphics design. All data were tested for distribution. All results were expressed as mean±SD. Multiple comparisons were made using one-way ANOVA followed by Tukey's post-test. Values of p<.05 were accepted as statistically significant.

## Results

No animal died during the experiment. Thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH) were assayed in testis homogenates. Total antioxidant power (TAP) was assayed in the blood plasma.

*Lipid peroxidation*

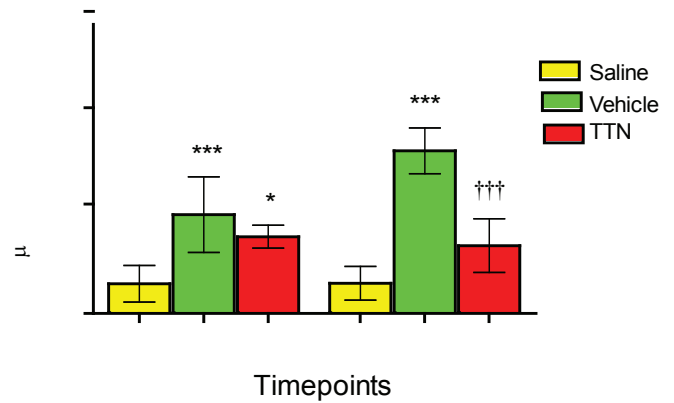
MDA concentrations decreased significantly ( $p < 0.001$ ) in vehicle and TTN groups in both timepoints. Additional decrease in MDA concentrations was seen in TTN rats compared with vehicle rats after three hours (T-3). (Figure 1)



**FIGURE 1** - Thiobarbituric acid-reactive substances concentrations (micromoles of malondialdehyde per gram of fresh tissue) in the testis of saline, DMSO and ternatin (TTN) pretreated rats. Bars represent mean  $\pm$  SD of control (yellow bars), vehicle (green bars) and TTN (red bars) groups at the end of the ischemia (T-0) and during reperfusion (T-3). \*\*\* $P < 0.001$ , vehicle and TTN groups are significantly different from control group by ANOVA test. †††  $P < 0.001$ , compared with vehicle group. T-0: before detorsion; T-3, 3 h after detorsion.

*Oxidative stress*

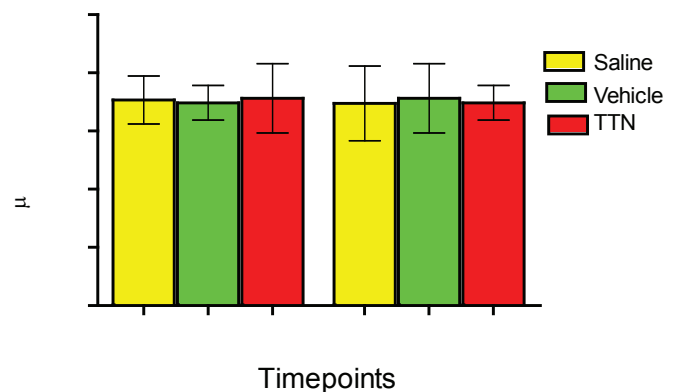
GSH concentrations increased significantly in vehicle ( $p < 0.001$ ) and TTN ( $p < 0.05$ ) at the end the ischemia (T-0). A significant increase in GSH concentrations was seen 3 hours after testis detorsion (T-3) in vehicle rats. GSH concentrations in saline and TTN treated rats were not different in T-3 timepoint (Figure 2).



**FIGURE 2** - Reduced glutathione (GSH) concentrations (micromoles of GSH per gram of fresh tissue) in testis homogenates of saline, DMSO and ternatin (TTN) pretreated rats. Bars represent mean  $\pm$  SD of control (yellow bars), vehicle (green bars) and TTN (red bars) groups at the end of the ischemia (T-0) and during reperfusion (T-3). Vehicle (\*\*\*) and ternatin groups ( $P < 0.05$ ) are significantly different from control group by ANOVA test. †††  $P < 0.001$  compared with vehicle group. T-0: before detorsion; T-3, 3 h after detorsion

*Plasma total antioxidant power*

Concentrations of TAP remained unchanged in vehicle and TTN groups. Antioxidants (Figure 3).



**FIGURE 3** - Plasma concentrations of total antioxidants (micromoles) in saline, DMSO and ternatin (TTN) pretreated rats. Bars represent mean  $\pm$  SD of control (yellow bars), vehicle (green bars) and TTN (red bars) groups at the end of the ischemia (T-0) and during reperfusion (T-3). Values were not different in T-0 and T-3 timepoints. T-0: before detorsion; T-3, 3 h after detorsion

**Discussion**

There is increasing evidence to suggest that toxic oxygen radicals play a role in the pathogenesis of ischemia/reperfusion injury in the testis. These free radicals react with lipid in cells and mitochondrial membranes forming lipid peroxides. Peroxidation of the lipid in membrane changes membrane permeability or disrupts

membrane and cell integrity. Lipid peroxidation is probably the most extensively investigated process induced by free radicals<sup>13</sup>.

Different therapeutic strategies have been investigated with the aim of reducing testis ischemia/reperfusion damage. Akgür *et al.*<sup>8</sup> submitted adult rats to ischemia from 1-5 hours followed by reperfusion (1-5 hours). Rats pretreated with allopurinol (200 mg/kg) did not show increase in lipid peroxidation. Turan *et al.*<sup>14</sup> studied the effects of vitamin E administered to adult Sprague-Dawley rats submitted to the torsion/detorsion of the testicle. Assessment of MDA showed that the vitamin E administered before or after testicular detorsion was innocuous<sup>14</sup>.

The lipid peroxidation inhibitory effects of several flavonoids such as luteolin, apigenin, galangin, gardenin D, (+)catechin<sup>15</sup>, quercetin<sup>16</sup> and rutin (quercetin-3-rhamnosyl glucoside)<sup>17</sup> have been previously reported. Pretreatment with ternatin enhanced higher antiperoxidative capacity of testis. This finding is verified by the significant decrease ( $P > .0001$ ) in MDA concentrations in ternatin pretreated animals when compared with DMSO and saline pretreated rats. The increase of GSH concentrations in TTN pretreated suggests that this flavonoid may attenuate the oxidative stress induced by I/R in rat testis immediately after detorsion. This protection is of short duration as this effect is not present after 3 hours. The use of TTN did not change plasma total antioxidants concentrations. This suggests TTN exerts no systemic antioxidant effects in rats submitted to T/D of the spermatic cord.

The data provides in vivo evidence of the antiperoxidative and antioxidative properties of TTN in torsted testis. This effect was increased by the antioxidative effect of the vehicle. DMSO protective effects in torsted testis has been demonstrated in one of our recently published paper dealing with this subject<sup>18</sup>.

## Conclusion

The results of the present study support the view that ternatin can exert a protective effect against testis tissue lipid peroxidation injury and oxidative stress caused by ischemia/reperfusion in rats subjected to T/D of the spermatic cord.

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