Tissues, Pathology, and Diagnostic Microscopy

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Protective effects of Caffeic Acid Phenethyl Ester through PI3K/AKT/mTOR pathway on ischemia-reperfusion damage in rat testicular tissue

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Testicular torsion is the most common genital trauma of the adolescent boy and has been implicated in testicular injury, altered hormone production, subfertility and infertility [1]. Testicular artery occlusion causes an enhanced formation of reactive oxygen species, which contributes to the pathophysiology of testis damage [2]. In this study, we aimed to investigate the effects of caffeic acid phenethyl ester (CAPE), an antioxidant and anti-inflammatory agent [1], in rats subjected to testicular torsion ischemia/reperfusion (I/R) damage and the effects on PI3K/AKT/mTOR pathway, which involved in cell survival [3].

Twentyone male rats (2 months, 200 gr weights) were divided into three groups: control group (n=7), testis torsion Ischemia/Reperfusion (I/R) group (n=7), I/R+CAPE group (n=7). Rats, except the control group, were subjected to left unilateral torsion (720º rotation in the clockwise direction) without including the epididymis (Fig.1A). After torsion (2 h) (Fig.1B) and detorsion (4 h) periods (Fig.1C), CAPE (10 µmol/kg, i.p.) was applied after 60 min after torsion I/R. Rats were sacrificed and orchidectomy was performed after application. Testicular tissue were fixed in Bouine's solution and they were then dehydrated and embedded in paraffin. Serial sections (thickness, 5 mm) were obtained, deparaffinized, and stained with hematoxylin-eosin (H&E). Light microscopy was performed without knowledge of the groups. Histological lesions were evaluated in both the tubular and extratubular compartments. Tissue samples were then analyzed via an avidin biotin peroxidase immunohistochemistry method. Anti-PI3K, anti-AKT and anti-mTORC-1 primary antibodies were used. Staining intensities were evaluated as mild, moderate and strong using semi-quantitative method. ANOVA statistical test was used to compare the results.

Testes of control group showed no changes in histological appearance either in the tubular and the extratubular compartments (Fig. 2A). On the other hand, histological observation following ischemia-reperfusion displayed interstitial effusion together with blood vessel dilation, loosening of Leydig cells, detachment of spermatogenic cells from the basement membrane and separation between the cells and disorganisation of seminiferous tubule cells were seen in the I/R testes (Fig. 2B). Administration of CAPE reduced the histological changes in testicular tissue (Fig. 2C).

Normal seminiferous tubule cells had moderate/moderate/strong PI3K, AKT and mTORC1 immunoreactivities in control group; mild/mild/moderate immunoreactivities were seen in I/R group and strong/moderate/strong immunoreactivities in I/R+CAPE treated groups, respectively (Fig. 2).

Our results demonstrate that CAPE treatment exerts a reperative and protective effects on testicular torsion I/R damage and part of this effect may be due to activating the PI3K/AKT/mTOR signal pathway. It was thought that these pathway may play an important role in testicular ischemia/reperfusion damage pathogenesis and CAPE might be preventive role in approach to the treatment of unilateral testicular torsion.

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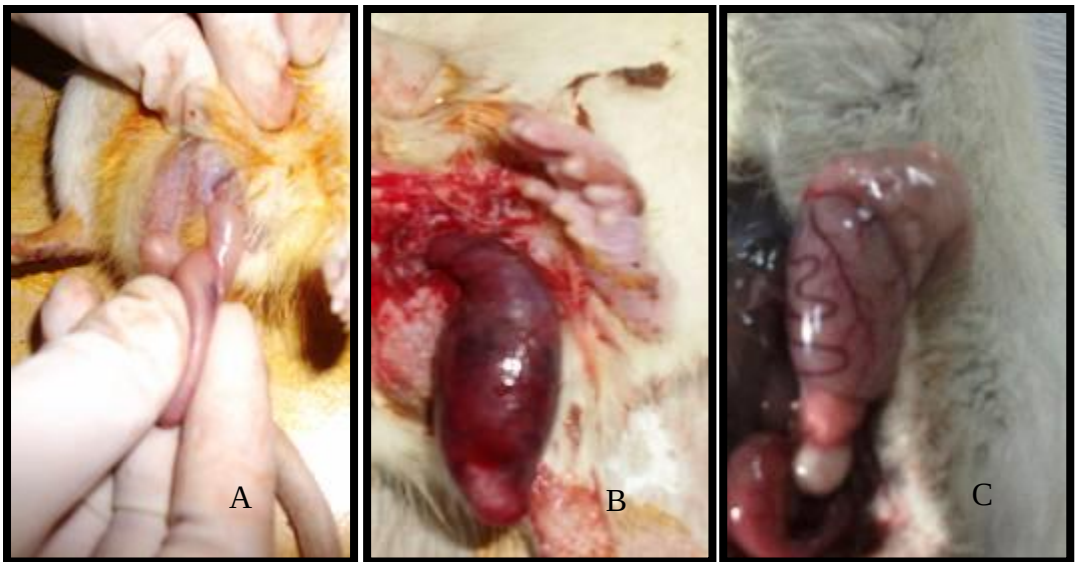

Figure 1. Photomicrographs of rat testis from Ischemia/Reperfusion Group (A), 2 Hour Torsion group (B), 4 Hour Detorsion group (C).

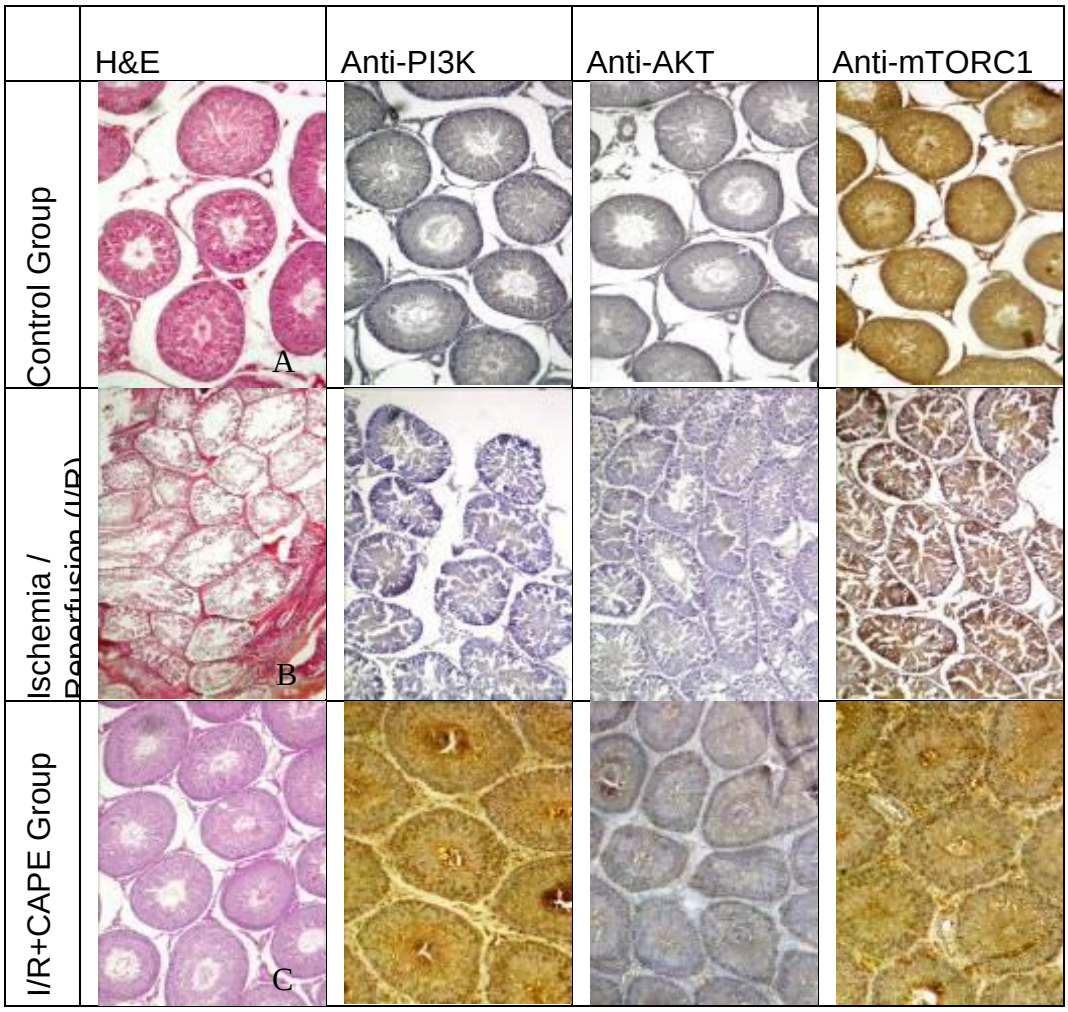

Figure 2. Immunohistochemical appearance of rat testis from control, I/R and I/R+CAPE Groups. Testicular damage in seminiferous tubules were seen in the I/R group. Immunoreactivities were seen as mild/mild/moderate in I/R group, while strong/moderate/strong in I/R+CAPE group respectively (X200).