Effects of *Potentilla fulgens* on Tuba Uterina in Ovariectomized Rats

Efectos de *Potentilla fulgens* en la Tuba Uterina de Ratas Ovariectomizadas

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**SUMMARY:** A total of 32 Wistar rats were divided into four equal groups: (I) sham, (II) ischemia, (III) reperfusion and (IV) *Potentilla fulgens*. In groups I and II, ovary torsion was not performed and no drug was administered. In group III, 1 h of ischemia and 2 h of reperfusion were performed and no drug was given. Group IV received 400 mg/kg/day *Potentilla fulgens* intraperitoneally 5 days before ischemia-reperfusion. All the parameters were observed to be significantly decreased (P<0.05) in all the experimental groups compared to the control group. In the sections of the ischemia-reperfusion group, degeneration of epithelium, dilation of blood vessels were observed. *Potentilla fulgens* administration reduced the morphological changes by induced I/R; in particular, infiltration, hemorrhage and vascular dilatation were decreased. *Potentilla fulgens* application during torsion, it plays an important role in maintaining the epithelial structure with E-cadherin expression. We suggest that PECAM-1(CD31) are a regulator of the microvascular response of the tubal mucosa.

**KEY WORDS:** *Potentilla fulgens*; Ischemia-reperfusion; Tuba uterina; Rat.

**INTRODUCTION**

*Potentilla fulgens* is an alpine plant of Western Himalayas which is consumed in all parts of the world for its promising medicinal properties. Pharmacologically, the aerial and root portions of the plant are reported to have antioxidant (*in vitro* models), antitumor, hypoglycemic and antihyperglycemic activities (Kaul *et al.*, 2011; Syiem *et al.*, 2003, 2009). The general signs and symptoms of toxicity, food and water intake and mortality rates of animals were observed within 72 h post-treatment. From these observations, LD50 was calculated using SPSS software (Chen *et al.*, 2005). The roots of the plant are used traditionally to treat ailments including gastric problems (peptic ulcers), mouth ulcers, diarrhea, gingivitis, diabetes, anthelmintic, cures pyorrhea and even improves gums (Kaul *et al.*, 2011). CD31 antigen (PECAM-1) is a single chain transmembrane glycoprotein with a molecular weight of 130 to 140 kD. The CD31 molecule is expressed on the surface of platelets, monocytes, granulocytes. The properties of CD31 antigen suggest that it is involved in interactive events during angiogenesis, thrombosis and wound healing (Leica Biosystems, 2016). E-cadherin is a calcium-dependent transmembrane protein involved in homotypic cell–cell interactions (Angst *et al.*, 2001). This adhesive molecule allows neighboring cells to stick together, it is also involved in the regulation of signaling events (Ozawa & Kemler, 1992; Aberle *et al.*, 1996). E-cadherin, epithelial cell differentiation and proliferation have been shown to act at the cell-cell connectivity.

In this study, we aimed to investigate the protective effects of *Potentilla fulgens* against the tuba uterina ischemia reperfusion (I/R) injury in rats by means of biochemical, histopathological, and immunohistochemical examination.

**MATERIAL AND METHOD**

The permission for the animal tests and experiments was given by the Animal Ethical Board of Dicle University Medical Faculty. Dicle University’s Experimental Animal Laboratory Institute supplied 30 healthy adult female wistar rats, weighing between 180 and 210 g. The rats were selected...
accounting to their estrous cycle. The rats were housed in plastic rat cages at 26±2 °C and they were exposed to 10–12 h of daylight. Animals were fed a standard laboratory diet and tap water ad libitum. A total of 32 Wistar rats were divided into four groups. The rats were first numbered randomly and then randomly divided into 4 equal groups: Sham, torsion, detorsion, and Potentilla fulgens groups. In group I (n= 8) sham, in group II ovary torsion was not performed and no drug was administered. In group III h of ischemia and 2 h of reperfusion were performed and no drug was given. In group IV, The Potentilla fulgens group received 400 mg/kg per day Potentilla fulgens intraperitoneally 5 day before Ischemia-reperfusion injury. Each rat was administered intramuscular ketamine hydrochloride (50 mg/kg ketamine hydroxide) and xylazine hydrochloride (10 mg/kg Rompun, Bayer Istanbul, Turkey) for anesthesia. The rats, except for in the sham-operated group, were subjected to right unilateral adnexial torsion which induced ischemia by occlusion of the tuba-ovarian vessels for 2 h. Rats in sham group were subjected to laparatomy only. In the torsion group, ovaries were surgically removed after 2 h of torsion. Right ovaries were surgically removed in all groups. The tuba uterina tissues were fixed in 10 % neutral buffered formalin solution for 24 h, dehydrated, cleared, and embedded in paraffin as usual. Serial tissue sections at a thickness of 4–5 mm were cut using the microtome and stained with hematoxylin and eosin (H&E).

**Immunohistochemistry.** Formaldehyde-fixed tissue was embedded in paraffin wax for further immunohistochemical examination. Sections were deparaffinized in absolute alcohol. Endogenous peroxidase activity was blocked with absolute methanol containing 0.4 % hydrochloric acid (1 M) and 0.5 % hydrogen peroxide (100 volumes) for 40 min at room. After washing in water followed by 0.05 M Tris-buffered saline, the sections were incubated in 1 % trypsin.

Fig. 1. A) The appearance showed in transvers section of tuba uterina (Control group) Mallory Azan stain Bar 100 µm. B) Degeneration in surface epithelium, thinning of connective tissue fibers extending to the surface epithelium, dilatation and hemorrhage in blood vessels (arrow) (Ischemia group) Mallory Azan stain Bar 50 µm. C) Loss in mucosal extension, separation in basal membrane, dilatation and hemorrhage in blood vessel (arrow) (Ischemia-reperfusion group) Mallory Azan stain Bar 100 µm. D) In the Ischemia-reperfusion+Potentilla fulgens partially preserved in mucosal extentions and basal membrane Mallory Azan stain Bar 100 µm.
After washing in cold water, staining was carried out as above, using E-cadherin (1/100), slides were incubated with ultra V-block and subsequently either with PECAM-1 (CD31) primary antibody used at a dilution of 1/100. A biotinylated secondary antibody was applied for one hour. Slides were then exposed to streptavidin peroxidase and chromogen. After each treatment, the slides were washed in PBS. The slides, having been counter stained with Mayer’s haematoxylin.

Statistical analysis. Statistical analysis was performed using commercially available software (SPSS v.10.0, SPSS Inc., Chicago, IL, USA). The difference between the groups for non-parametric data with the Kruskal-Wallis test, two group comparisons Bonferonni correction was made after using the Mann-Whitney U test.

RESULTS

All the parameters were observed to be significantly decreased (P <0.001) in all the experimental groups compared to the control group.

The tubas uterinas of the ischemia groups showed some cellular hypertrophy and hyperplasia of the columnar epithelium. In the ischemia group, vascular dilatation and congestions were observed in tunica mucosa and sub epithelial area, the epithelial surface and extending to the reduction in cilia structures was decreased. In the sections of the ischemia-reperfusion group, degeneration of epithelium, dilation of blood vessels were observed. Potentilla fulgens administration reduced the morphological

Fig. 2. A) Control group: E-cadherin positive expression in the cell apical cilia connections (arrow), E-cadherin immunstaining Bar 50 µm. B) In the ischemia group, Decomposition and degradation of the surface epithelial cell-cell connections(arrow), weak expression of E-cadherin in apoptotic cells, E-cadherin immunstaining Bar 50 µm. C) In the ischemia-reperfusion group, Degeneration and picnosis in the surface epithelium, breaking the cell-cell connections, E-cadherin immunstaining Bar 50 µm. D) In the ischemia-reperfusion + Potentilla fulgens group Cilia and the cell-cell connections regularly (positive E-cadherin expression), light leaving the basal part of the mucosal folds, E-cadherin immunstaining Bar 50 µm.
changes by induced I/R; in particular, infiltration, hemorrhage and vascular dilatation were decreased.

In the ischemia group, the positive reaction inflammatory cells in sub epithelial and the blood vessels in the stromal region of CD31 expression was observed. As a result of ischemia, in which blood vessels dilate to fulgens treated group was observed near normal appearance.

**DISCUSSION**

Torsion of the ovary or tuba uterina (adnexal torsion) is an uncommon event. Rotation of the infundibulopelvic ligament causes compression of the ovarian vessels and impedes lymphatic and venous outflow and arterial inflow. Ovarian torsion occurs due to the rotation of the adnexa. The degree of rotation and duration of torsion determine the severity of the injury (Inderdeo et al., 1996).

MDA (Malondialdehyde) is a secondary product of oxidative stress formed during lipid peroxidation and is significantly increased by I/R injury. It is released as a result of toxic effects of active oxygen species which destroy unsaturated fatty acids in the cell membrane (Melchiorri et al., 1995). In the present study, the levels of ovarian MDA were significantly decrease by I/R injury. Tunç et al. (2014) as the result of ovarian torsion, in the tuba uterina epithelial cilia loss, piknosis nucleus, while the submucosa showed inflammation. Torsion due to lack of blood flow to the inability of the ovaries. In the epithelium of the tuba uterina it has caused the reduction of degeneration and delete it. We observed a typical cells with nuclei, congestion in blood vessels and inflammatory cell infiltration in the sections of tuba uterina in torsion group. In the present study, *Potentilla fulgens* prevented the hemorrhage and degree of vascular dilatation, and congestion. These effects of *Potentilla fulgens* may occur through its antioxidant properties.
E-cadherin is an important molecule in the maintenance of epithelial integrity (Takeichi, 1990) Damage to the epithelium may result in loss of E-cadherin membrane expression and intercellular contacts (Nawijn et al., 2011).

In the torsion group, the expression of E-cadherin in epithelial degenerations was localized weakly in the intercellular border. In torsion + *Potentilla fulgens* group, E-Cadherin was expressed tuba uterina the whole surface of polarized epithelial cells. We observed an association between the expression of E-cadherin in tubal surface epithelium and the morphology of cells, with expression of E-cadherin predominant in ciliar columnar cells. *Potentilla fulgens* application during torsion, it plays an important role in maintaining the epithelial structure with E-cadherin expression. In the torsion group, PECAM-1 expression showed positively vascular endothelial cells in submucosa, in addition PECAM-1 showed a significant increase in inflammatory cells

It is an important marker on an endothelial mitogen PECAM-1 (CD31) angiogenesis regulation. We suggest that PECAM-1 (CD31) are a regulator of the microvascular response of the tubal mucosa.

**REFERENCES**


**RESUMEN:** Un total de 32 ratas Wistar fueron divididas en cuatro grupos: (I) Sham, (II) isquemia, (III) reperfusion y (IV) *Potentilla fulgens*. En los grupos I y II, no se realizó la torsión de ovario y ni se administró ningún tipo de fármaco. En el grupo III, se produjo isquemia por 1 h seguido de reperfusion por 2 h (I/R), sin administracion de fármacos. El grupo IV recibió 400 mg/kg por día de *Potentilla fulgens* vía intraperitoneal durante cinco días previo al protocolo de isquemia-reperfusión. Se observó que todos los parámetros disminuyeron significativamente (P <0,05) en todos los grupos experimentales en comparación con el grupo control. En las secciones del grupo de isquemia-reperfusion, se observó degeneración del epitelio y dilatación de los vasos sanguineos. La administración de *Potentilla fulgens* reduce los cambios morfológicos inducidos por I/R; en particular, la infiltración, la hemorragia y la dilatación vascular. La aplicación de *Potentilla fulgens* durante la torsión, desempeña un papel importante en el mantenimiento de la estructura epitelial con la expresión de E-cadherina. Sugerimos que PECAM-1 (CD31) es un regulador de la respuesta microvascular de la mucosa tubárica.

**PALABRAS CLAVE:** *Potentilla fulgens*; Isquemia-Reperfusion; Tuba uterina; Rata.

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