

Studi Bioprospeksi Efek Krioprotektif Madu Lengkek (*Dimocarpus longan*) terhadap Integritas Struktur Folikel Preantral dan Profil Protein Apoptosis Jalur Intrinsik Pasca Vitrifikasi Ovarium Tikus (*Rattus norvegicus* L.) Galur Sprague Dawley = Bioprospecting Study of Cryoprotective Effects of Longan Honey (*Dimocarpus longan*) on Preantral Follicle Structure Integrity and Protein Expression Profile of Intrinsic Pathway Apoptotic Post Ovarian Vitrification of Rat (*Rattus norvegicus* L.) Sprague Daw

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Abstrak

Telah dilakukan penelitian untuk mengidentifikasi efek krioprotektif madu lengkek (*Dimocarpus longan*) terhadap integritas struktur folikel preantral dan profil ekspresi protein apoptosis jalur intrinsik. Sebanyak 24 ekor tikus (*Rattus norvegicus* L) dikelompokkan menjadi 8 kelompok, terdiri atas KKN, KKV (NaCl 0,9%), KKP1 (EG 7,5%), KKP2 (EG 15%), KP1 (EG 7,5% + ML 7,5%), KP2 (EG 7,5% + ML 15%), KP3 (EG 15% + ML 7,5%), dan KP4 (EG 15% + ML 15%). Pengamatan terhadap densitas, struktur folikel serta ekspresi protein Bax, Bcl2 dan Caspase3 dilakukan terhadap sayatan ovarium yang dibuat dengan metode parafin dengan pewarnaan Hematoksilin-Eosin (HE) dan imunohistokimia. Antibodi primer yang digunakan adalah antibodi poliklonal Rabbit Anti-Bax (A00183 Boster, USA), Rabbit Anti-Bcl-2 (A00040-2 Boster, USA) dan Active Caspase-3 Rabbit Polyclonal Antibody (ab4051 Abcam, UK) dan One Step Neopoly Detection System Kit (BGNK-0025 Biogear, USA). Identifikasi terhadap tiap tipe folikel preantral menggunakan mikroskop cahaya yang terhubung dengan perangkat lunak Image Raster dan IHC profiler. Hasil penelitian menunjukkan, efek krioprotektif madu lengkek dapat meningkatkan densitas folikel, indeks folikel intak G2 dan G3, menurunkan indeks folikel G1, menekan ekspresi protein Bax dan caspase 3 serta meningkatkan ekspresi protein Bcl2. Dengan demikian, madu lengkek memiliki potensi untuk dikembangkan sebagai krioprotektan ekstraselular alami dalam aplikasi vitrifikasi ovarium.

.....A study was conducted to identify the cryoprotective effect of longan honey (*Dimocarpus longan*) on the structural integrity of the preantral follicle and the expression profile of the intrinsic pathway of apoptosis protein. A total of 24 rats (*Rattus norvegicus* L) were grouped into 8 groups, consisting of KKN, KKV (NaCl 0.9%), KKP1 (EG 7.5%), KKP2 (EG 15%), KP1 (EG 7.5% + LH 7.5%), KP2 (EG 7.5% + LH 15%), KP3 (EG 15% + LH 7.5%), and KP4 (EG 15% + LH 15%). Observations on the density, follicular structure and protein expression of Bax, Bcl2 and Caspase3 were carried out on ovarian sections made by paraffin method with Hematoxylin-Eosin (HE) staining and immunohistochemistry. The primary antibodies used were Rabbit Anti-Bax polyclonal antibody (A00183 Boster, USA), Rabbit Anti-Bcl-2 (A00040-2 Boster, USA) and Active Caspase-3 Rabbit Polyclonal Antibody (ab4051 Abcam, UK) and One Step Neopoly Detection System Kit (BGNK-0025 Biogear, USA). Identification of each type of preantral follicle using a light microscope connected to Image Raster software and an IHC profiler. The results showed that the cryoprotective effect of longan honey could increase follicle density, G2 and G3 intact follicle index, decrease G1 follicle index, suppress Bax protein expression and caspase 3 and increase Bcl2 protein expression. Thus, longan honey has the potential to be developed as a natural extracellular cryoprotectant in

ovarian vitrification applications.