

## **EFEKTIVITAS VCD (4-VINYL CYCLOHEXENE DIEPOXIDE) SEBAGAI BAHAN ANTIFERTILITAS TIKUS SAWAH (*Rattus argentiventer*, Rob and Kloss) BETINA PADA SKALA LABORATORIUM**

### **INTISARI**

Penelitian mengenai efektivitas VCD sebagai bahan antifertilitas pada tikus sawah betina dilakukan dengan tujuan: Mempelajari pengaruh ovotoksik VCD pada tikus sawah betina baik secara 1) *in vivo* maupun 2) *in vitro* dan pengaruh spesifiknya terhadap folikel *primordial*; 3) Mempelajari kemampuan *ovarium* tikus sawah sebagai organ target dalam merespon senyawa VCD; 4) Mendeteksi efek ovotoksik VCD terhadap perkembangbiakan tikus sawah melalui formulasi umpan pada skala laboratorium dan *enclosure*. Kegiatan A dilakukan dengan mencekok 37 ekor tikus sawah betina dewasa selama 16 hari berturut-turut yang dibagi menjadi tiga kelompok (kontrol, N=15; dosis 500 mg VCD/kg berat badan (BB), N=13; dan dosis 750 mg/kg BB, N=9. Kegiatan B dilakukan dengan mengkultur *in vitro ovarium* cindil *Post Natal Day 4* (PND4) (n=10) dalam medium DMEM/Ham's F-12 yang dicampur VCD (30  $\mu$ M) selama 6 hari. Sediaan histologi *ovarium* kedua kegiatan tersebut dibuat dengan metode parafin dan pewarnaan *Hematoxylin Eosin* (HE). Kegiatan C meliputi deteksi keberadaan *microsomal Epoxide Hydrolase* (mEH) sebagai enzim pendetoksifikasi VCD, diawali dengan kultur *in vitro ovarium* cindil PND3, PND5 dan PND8 (n=14) dan deteksi dilakukan secara kualitatif dengan IHC. Kegiatan D diawali dengan uji umpan VCD terhadap tikus sawah betina dewasa di laboratorium pada variasi rentang waktu (5, 10, dan 15 hari). Individu uji selanjutnya dipasangkan dengan pejantan non-perlakuan (6 betina : 2 jantan) pada plot berukuran 1,25 m x 3 m dalam *enclosure*. Analisis data dilakukan dengan uji ANOVA dan dilanjutkan dengan LSD. T-test digunakan untuk mengetahui perbedaan pada data yang berpasangan. Pengamatan keberadaan enzim mEH dilakukan secara deskriptif terhadap gambaran imunohistokimia *ovarium* hasil IHC. Hasil kegiatan A menunjukkan bahwa VCD menyebabkan penurunan jumlah folikel *primordial* secara nyata antar perlakuan ( $p < 0,05$ ). Perubahan berat organ secara nyata hanya terdeteksi pada *glandula adrenalis*, sedangkan berat organ lainnya (*ovarium*, *uterus*, *hepar*, *pulmo*, *cor*, *ren*, *glandula adrenalis* dan *lien*) tidak menunjukkan perbedaan secara signifikan ( $p > 0,05$ ). Hasil analisis statistik kegiatan B menunjukkan bahwa jumlah folikel *primordial* berbeda nyata ( $p < 0,05$ ) antara perlakuan dan kontrol, dengan penurunan sekitar 37% pada individu perlakuan. Respon imunoreaktif terhadap VCD pada kegiatan C dicirikan dengan bulatan berwarna coklat kekuningan dan cenderung meningkat seiring dengan bertambahnya umur *ovarium*. Hal ini mengindikasikan bahwa *ovarium* mampu merespon VCD melalui keterlibatan mEH sebagai enzim pendetoksifikasinya. Hasil kegiatan D menunjukkan bahwa efek pemberian umpan VCD terhadap perkembangbiakan dan populasi akhir belum muncul. Lebih dari 50% individu pada semua plot mengalami kebuntingan dan rata-rata jumlah embrio tidak berbeda nyata ( $p > 0,05$ ). Individu baru ditemukan pada semua plot dengan total

jumlah cindil dan populasi akhir menunjukkan pola yang sama. Secara keseluruhan hasil penelitian ini menunjukkan bahwa VCD berpotensi menurunkan jumlah folikel primordial baik secara *in vivo* maupun *in vitro*. *Ovarium* mampu merespon VCD dengan terdeteksinya mEH sebagai enzim pendetoksifikasi senyawa tersebut. Efek toksik VCD terhadap perkembangbiakan tikus belum muncul dalam kurun waktu satu bulan setelah perlakuan pengumpanan VCD selama 5-15 hari.

Kata kunci: tikus sawah, VCD, antifertilitas, folikel primordial, enzim mEH

**THE EFFECTIVENESS OF VCD (4-VINYL CYCLOHEXENE  
DIEPOXIDE) AS AN ANTIFERTILITY AGENT ON FEMALE RICE  
FIELD RAT (*Rattus argentiventer*, Rob & Kloss)  
AT LABORATORY LEVEL**

***Abstract***

A study on the effectiveness of VCD as an antifertility agent for the female rice field rat has been conducted. The goals of this study are: to study the ovotoxic effect of VCD on female rice field rat both 1) *in vivo* and 2) *in vitro* and its specific effects to the primordial follicles; 3) to study the capability of rice field rat ovary as a target organ on responding to the VCD; 4) to detect the ovotoxic effect of VCD to the rice field rat breeding through providing a pellet mixed with VCD in the laboratory and continued with mating trial in an enclosure. Activity A was conducted by orally gavaged 37 of adult female rice field rats for 16 consecutive days. The animals were divided in three groups as control (N=15), dosages of 500 mg/kg BW (N=13) and 750 mg/kg BW (N=9). The activity B was conducted by culturing the PND4 ovaries (n=10) *in vitro* in DMEM/Ham's F-12 medium mixed with VCD (30  $\mu$ M) for six days. All ovaries from both activities were stained using Hematoxylin Eosin technique. The activity C was detection of mEH as detoxifying enzyme of VCD in and preceded by culturing *in vitro* of PND3, PND5 and PND8 rice field rat ovaries. The ovaries were then used as samples for the enzyme detection qualitatively by IHC. Adult female rice field rats in activity D were introduced with pellet mixed with VCD in the laboratory for different time periods (5, 10 and 15 days), consecutively. These animals were then released to an enclosure and mated with untreated males with sex ratio 6 females : 2 males in each plot (1.25 x 3 m). ANOVA was performed to analyze the data and continued with LSD test if there is a significant difference. Immunoreactive response of the ovary was observed descriptively to identify the presence of mEH. Results from activity A indicate that VCD significantly decreased primordial follicle number ( $p < 0.05$ ). There was no significant change of organ weights except the adrenal glands. The number of primordial follicles of the ovaries from *in vitro* culture in activity B was differed significantly ( $p < 0.05$ ). The treated ovaries have follicles 37% lower than that of the control. Immunoreactive responses from IHC in activity C was detected as yellowish-brown dots and tends to increase as the ovaries get older. This indicates that the ovary has mEH as a detoxifying enzyme for countering VCD. The effect of VCD-pellet in activity D was not detected yet which accounted for one month after 5-15 days pellet treatment in the laboratory. More than 50% of animals from all plots got pregnant and the average of embryo number was not significantly different. ( $p > 0.05$ ). Some litters were found in all plots at the end of mating trial. The total number of litters and population indicate the similar pattern in all plots. In general, VCD was potentially decreased the primordial follicle number both *in vitro* and *in vivo*. The presence of mEH indicated that the ovary was able to detoxify the compound. The

toxic effect of VCD to the female rice field rat breeding was not detected yet after one month of 5-15 days pellet treatment in the laboratory.

Keywords: rice field rat, VCD, antifertility, primordial follicle, mEH

