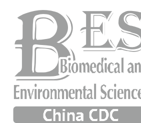
Letter to the Editor



Evaluation of the Effects of Cypermethrin on Female Reproductive Function by Using Rabbit Model and of the Protective Role of Chinese Propolis

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The prophylactic effects of Chinese propolis against cypermethrin toxicity were evaluated by performing ovary and uterus histopathology, as well as by characterizing ovarian function, embryos, and litters. Cypermethrin induced atypia in the ovary and uterus, and decreased the ovulation sites and the number of embryos. Cypermethrin-induced oxidative stress during pregnancy, decreased the parturition rate as well as the number and weight of offspring and increased the incidence of morphological malformations in the offspring. Administration of propolis to cypermethrin-treated animals mitigated cypermethrin-induced reproductive toxicity.

Cypermethrin is a synthetic form of the naturally derived insecticide pyrethrin, and is used extensively worldwide. It degrades in soil and plants within a few days; however, its concentration stays relatively constant after a treatment indoors, and it can cause hazardous health effects. Cypermethrin can affect reproduction-related steroids, and crosses the placental barrier to interfere with fetal development[1]. Recent studies have shown that the reproductive toxicity of cypermethrin can be partially mediated by oxidative stress[2].

Propolis is a mixture of resinous plant substances that are produced by honeybees. It contains an abundance of phytochemicals (flavonoids, phenolic acids, and long-chain fatty acids) with antioxidant activities[3]. Therefore, this study aimed to identify the effects of cypermethrin on the different reproductive functions of female rabbits and their offspring, and the protective role of propolis based on its chemical constituents.

The chemical constituents of the ethanolic extract of propolis (Guang Zhou Herb & Bee Products Co., Ltd., Tianhe District, Guangzho, China) were identified by gas chromatography-mass spectrometry (GC/MS). A Thermo Scientific TRACE-1300 series GC system fitted with a fused silica DB-5 capillary column (inner diameter, 30 m × 0.32 mm; film thickness, 0.25 µm), coupled to a Triple Quadrupole Mass (TSQ 8000 Evo) was used (Thermo Fisher Scientific Inc., Austin, Texas, USA). The column temperature was set at 40 °C with an initial hold of 5 min, which was then increased to 270 °C at 2 °C/min, and maintained at 270 °C for 20 min. The splitless injection mode was used (0.5 µL of a 1:1000 methanol solution). The carrier gas was helium with a flow rate of 1.0 mL/min. Injector and detector temperatures were 250 °C and 290 °C, respectively. Mass spectra were scanned in the range of 40-700 amu, and the scan time was 5 scans/s. The constituents were identified based on a combination of retention index data and mass spectral data using the Wiley 9 library.

Forty female V-line rabbits (5 months old, weighing 2.935±0.029 kg), obtained from the Laboratory of Rabbit Physiology Research, Faculty of Agriculture, Alexandria University, Egypt, were used. The rabbits were handled in accordance with the Standard Guide for the Care and Use of Laboratory meeting the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes. Propolis (50 mg/kg body weight) and alpha-cypermethrin (50 mg/kg body weight; 1/5 lethal dose, LD_{20}) were dissolved in corn oil. Alpha-cypermethrin [α-cyano-(3-phenoxyphenyl)methyl(±)-*cis*/*trans*-3-(2,2-dichlorovinyl)2,2-dimethylcycloprop-anecarboxylate] is a racemic mixture of two isomers of cypermethrin with the molecular formula $C_{22}H_{19}C_{l2}NO_3$ and molecular weight of 416.3 g/mol (Chimac Agriphar S.A., Belgium). Female rabbits were randomly divided into four groups (*n*=10), and administered corn oil (Con group), propolis (Pro

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group), cypermethrin (Cyp group), or their combination (Cyp/Pro group) by oral gavage. Each female rabbit was treated with 25 IU of equine chorionic gonadotropin (Gonaser®, Hipra, Spain) 2 d before being allowed to naturally mate with fertile male rabbits. Female rabbits of each group were divided into two subgroups (5 females each). The first subgroup received the treatments for 60 d, and allowed to mate 2 d prior to being euthanized. Reproductive organs were directly removed and weighed after the animals were euthanized. The number of ovulation points on each ovary was recorded. Excised oviducts were flushed with phosphate buffer solution containing 20% bovine serum albumin (Sigma, USA). Next, the collected embryos from each oviduct were counted and their developmental stage was recorded. Finally, the ovaries and uteri were fixed and processed for preparation of histological sections. The second subgroup received the same treatments immediately after mating until parturition for two gestation periods. The parturition rate, abortion rate, and offspring characterization data were recorded.

Blood samples of the first subgroup were collected on the day of mating to determine the plasma estradiol (E_2) concentration. In the second subgroup, blood samples were obtained on days 14 and 28 after mating. The activities of glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase (CAT) (Reactivos GPL, Barcelona, Spain) and concentrations of malondialdehyde acetate (MDA; Biodiagnostic, Giza, Egypt) and progesterone (P_4) were determined. Hormonal assessment was carried out using commercial solid-phase enzyme immuno-assay kits (DRG International Inc., Springfield, USA).

Results are expressed as mean±standard error values. Analyses of variance (ANOVA) were conducted to determine significant differences among groups, followed by Duncan's new multiple range test. Data expressed as percentages were analyzed using a Chi-square test. Statistical significant was considered at $P<0.05$. All statistical analyses were carried out using the Statistical Analysis System program (SAS Institute, 2001, Version 8. Cary, USA).

The analysis of the propolis ethanolic extract helped identify 17 chemical compounds including alkaloids such as 3,3-dimethyl-2-phenyl-2-(1-oxo-1, 2,3,4-tetrahydronaphthalen-2-yl) azirane (21.40%), N,N-dimethyl deacetyl colchinine (3.67%), 2,4-bis (4-chlorophenyl)-5,6-dihydrobenzo[h] quinazoline (3.02%); flavones such as lucenin 2 (5.17%), baicalin (3.82%), and quercetin 7,3',4'-trimethoxy ester (2.71%); and organosilicons such as cyclohexasiloxane, dodecamethyl (6.51%) and hexasiloxane,1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl (5.41%) (Table 1).

Table 1. Retention Times (RTs, minute) and Percentages of Relative Area (%) of Chemical Constituents of Propolis Ethanolic Extract Detected by Gas Chromatography-mass Spectrometry (GC/MS)

RT	Area	Compound
2.02	21.4	3,3-Dimethyl-2-phenyl-2-(1-oxo-1,2,3,4-tetrahydronaphthalen-2-yl) azirane
7.41	2.94	Cyclopentasiloxane, decamethyl-(CAS)
10.04	6.51	Cyclohexasiloxane, dodecamethyl
12.32	5.41	Hexasiloxane,1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl
27.67	3.70	Glycodeoxycholic acid
27.95	7.59	Cholestan-3-one, cyclic1,2-ethanediyl aetal, (5á)-(CAS)
28.01	3.67	N,N-Di methyl deacetyl colchinine
28.73	3.02	2,4-Bis(4-chlorophenyl)-5,6-dihydrobenzo[h]quinazoline
29.02	5.36	5-Chloro-3-(3,4-dimethoxyphenyl)-6-methyl-2H-1,4-oxazin-2-one
29.89	2.71	Quercetin7,3',4'-Trimethoxy ester (CAS)
30.11	2.47	6-Ethyl-5-(4'-trifluoro methyl phenyl) pyrimidine-2,4-diamine
30.19	3.82	Baicalin
31.93	5.17	Lucenin 2 (Luteolin 6,8-C-diglucoside)
32.04	3.85	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxan
34.53	5.82	3,9-Epoxypregnane-11,14,18-triol-20-one,16-cyano-3-methoxy-,11-acetate,16-cyano-3-methoxy-,11-acetate
34.80	4.23	6-Amino-5-cyano-4-(5-cyano-2,4-dimethyl-1H-pyrrol-3-yl)-2-methyl-4H-Pyran-3-carboxylic acid ethyl ester
34.89	5.77	7-Hydroxymethyl-1-bromo-4-isopropoxy-5-methoxyna phthalene

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