Original Article

Propolis Attenuated the Toxicity of Methomyl on Rat Fetuses and Pups During Pregnancy and Lactation

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ABSTRACT

Introduction: The global use of methomyl has raised concerns about its potential adverse effects, particularly during pregnancy. Propolis has received a lot of attention lately because of its possible therapeutic benefits and ameliorative effects, particularly during pregnancy.

Objectives: The current double-approach study sets out to assess the possible impact of ma-ternal methomyl injection on rat fetuses and pups' morphology and endoskeletal development, as well as rat dams' and pups' chromosomal abnormalities. Furthermore, we looked at the po-tential protective properties of propolis against methomyl-induced toxicity.

Methods: Oral administration of methomyl (2 mg/kg) and propolis (300 mg/kg) were given to pregnant (from 6th to 15th days of pregnancy) and lactating rat mothers (from 1st to 14th post-natal days). The rats were divided into the following groups: 1) the control group receiving 1ml distilled water, 2) propolis group receiving 1ml propolis; 3) methomyl group receiving 1ml methomyl; and 4) combination group receiving 1ml methomyl and 1 ml propolis. A total of 48 females, 208 fetuses and 152 pups were utilized in the current study.

Results: Maternal methomyl administration induced many morphological and endoskeletal abnormalities, such as umbilical hernia, small ear pinna, soft membranous skin, subcutaneous hemorrhage, hypognatha and kyphosis. In addition to histological changes in bone sections of rat pups and decreased cortical ossification. In addition, methomyl induced a significant increase in the frequency of aberrant chromosomes, which included fragmentation, ring chromosomes, centric attenuation, centric fusion, end to end association and chromosome gaps. Interestingly, propolis administration evidently ameliorated and reversed the majority of the drastic effects of maternally administered methomyl on rats' prenatal and postnatal development.

Conclusions: Our results reported that during lactation and pregnancy propolis could be used in the form of dietary supplement to help improve the hazard effects of maternal administra-tion of methomyl.

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Key Words: Chromosomal aberrations; endoskeleton; methomyl; ossification; propolis.

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INTRODUCTION

The term "pesticides" refers to natural or artificial chemical substances that are employed to eradicate undesired plants, fungi, insects, and rodents[1]. The increasing use of pesticides harms ecosystems and public health, even if its primary purposes include crop protection, food material preservation, and the avoidance of vector-borne diseases[2]. Numerous health issues, including reprotoxic consequences such stillbirth, infertility, low birth weight, premature birth, ovarian defects, and changes in sexual behavior, are brought on by human exposure to pesticides[3]. In terms of structure and mechanism, carbamates are a class of insecticides that are related to organophosphate insecticides that are made from carbamic acid[4,5]. Aldicarb, pirimicarb, carbofuran, oxamyl, ethinenocarb, carbaryl, trimethacarb, fenobucarb, methomyl, and propoxur are common carbamates linked to harmful exposure[6].

According to numerous reports, pregnant rats exposed to pesticides at the organogenesis stage have developmental toxic consequences such as skeletal, soft tissue, and morphological abnormalities[7]. Introduced by DuPont in 1966, methomyl (Lannate1) is a carbamate insecticide that has anticholinesterase activity[8]. One of the most significant carbamates that is widely utilized globally is methomyl[9]. Chronic exposure to methomyl has also been shown to have a direct harmful impact on the female reproductive system, causing alteration of reproductive hormones and altering implantation locations, fertility index, and fetal weights[10]. Progesterone levels, ovary relative weights, litter size, sex ratio, gestation index, and newborn mean weight all significantly decreased after methomyl injection[11]. Furthermore, methomyl injections in pregnant rats resulted in decreased food intake and body weight gain, lower neonatal weights, and changes in the sex ratio, according to Rachid *et al*.[12]. Furthermore, Zedin and Galal[13] discovered that the male albino rats' bone marrow cells had chromosomal abnormalities caused by malathion insecticide. It has recently been demonstrated that methomyl causes DNA damage and modifies the adrenal-gland's development in the fetuses, as well as the postnatal pups[14].

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A potential source for the development of novel pharmaceuticals is natural products[15]. Because apiculture products have been shown to improve human health, a number of studies on their application in phytotherapy and diet have been published in recent decades[16]. Bees (Apis mellifera L.) are the primary producers of propolis, a chemical made primarily from plant-based basic materials[17]. In several nations, it is utilized as a traditional herbal remedy and as bee adhesive for damaged open places in hives[18]. Propolis is said to include about 300 different chemical constituents, including carbohydrates, vitamins, minerals, amino acids, and essential and aromatic oils[19]. Because the exudate that bees collect contains secondary plant metabolites, propolis is used in natural medicine to treat a variety of ailments and demonstrates biological activities[20,21]. The metabolites that exhibit antioxidant activity and may function as reducing and stabilizing agents of nanoparticles include phenolic ethers, phenolic acids, alcohols, hydrocarbons, aromatic and aliphatic acids, ketones, aldehydes, fatty acids, flavonoids, esters and their derivatives, amino acids, terpenoids, lignans, sugars, minerals, vitamins, artepillin C, and cinnamic acids[22]. A wide range of biological activities are exhibited by propolis, such as antifungal[23], antibacterial[24], antiviral[25], antioxidant[26-28], anticancer, and preservative properties[29-32]. Propolis treatment also improved the body weight of fetuses and corrected skeletal abnormalities brought on by caffeine induction in nursing mothers[33]. The administration of honey was shown by Shahrour *et al*.[34] to boost the frequency of chromosomal abnormalities in rats. Thus, the current work employs a two-pronged strategy to examine the harmful effect of methomyl administration in aperiod of pregnancy and nursing from morphology, ossification of the endoskeleton, and chromosomal abnormalities of rat prenatal and postnatalpups. Furthermore, the goal is to investigate if propolis can prevent these harmful effects and whether it can be taken as a dietary supplement when a woman is pregnant or nursing.

METHODS

Animals and grouping

The Vacsera animal breeding farm provided healthy adult male and female Wistar albino rats (Rattus norvegicus) that were 8±1 weeks old and weighed an average of 180±15g. The animals were kept in a 24±2ºC room with a 12:12 light/dark cycle. Rats were given access to water and food[35]. Overnight, breeding at a ratio of two females to one male was accomplished. Vaginal smears were examined in the early morning hours to check for the presence of sperm, which verified successful mating. Females that tested positive for sperm were categorized as being in gestational day zero (GD0) and kept in separate plastic cages. The current study utilized 72 rats (24 males and 48females), 208 fetuses & 152 pups. There were four groups of six healthy pregnant rats in each:

Control group, 1ml of distilled water was orally administered.

Propolis group, 1ml of propolis was orally administered.

Methomyl group, 1ml of methomyl was orally administered.

Combined group, 1ml of methomyl, then 1ml of propolis, an hour later.

The treatment began every day for 10 days, from six gestational day to the 15th GD during pregnancy. The rats' mothers and also their pups were put separately for the postnatal experiment, and the administration began on postnatal day (PD) 1 and continued until PD 14.

Chemicals

Propolis

Faculty of Agriculture Bee Products Research Unit kindly provided propolis powder. The method described by Usman *et al*.[36] was used to make the propolis ethanolic extract. Briefly, the frozen propolis was ground and 30 g propolis was dissolved in 100 ml of 70% ethanol at room temperature. The mixture was then mixed vigorously and incubated in a dark room for one week at 37°C, and centrifuged at 5000 rpm for 10 min. The supernatant was then filtered. The extract was then freeze-dried to remove the ethanol and water. The dried propolis extract was stored at –20°C. Pregnant rats were given 300 mg/kg of an aqueous solution of propolis orally.

Methomyl

The pure blue crystal powder known as Lannate-90® insecticide with 10% inert carrier components was supplied from Starchem Industrial-Shoura Chemicals and was dissolved directly in distilled water, administered with a dose of 2 mg/kg[10].

Embryo collection& embryotoxicity estimation

On GD 20, pregnant females underwent weight measurements, halothane inhalation anesthesia, and sacrifice. The following data was collected after the uterine horns were removed, dissected, photographed, and examined: the number of live and dead embryos, the number of implantation and resorption sites, the fetal body weight, the length of the crown-rump, the placental weight, and the gross outward modifications of the fetus. The placental index, resorption rate, fetal viability, and post-implantation loss were determined by analyzing the collected data. After the embryos were dissected and preserved in 10% neutral buffered formalin, the skeletons were stained with Alizarin red S and Alcain blue for any potential endoskeletal abnormalities.

Morphometric parameters

The resorptions (the number of absorbed embryos), resorption rate (%) ((number of absorbed embryos × 100)/(number of alive embryos), fetal viability (%) ((number of dead embryos × 100)/(number of alive embryos)), fetal weight (g), fetal length (cm), placental weight (g), placental index (fetal body weight)/(placental weight) and fetal morphological malformation were recorded.

Endoskeletal investigation

Using the osteogenic indicator Alzarin red S for bone and the chondrogenic indicator Alcian blue for cartilage, a double staining transparency technique was used for endoskeletal preparation. This was accomplished by using a technique that was first presented by Cortés-Delgado *et al*.[37] then modified by Badawy *et al*.[38]. The Heerbrugg M3C dissection microscope was used to view the dyed fetuses in glycerol at low resolution, and the Letiz Laborlux S light microscope was used for high resolution examination. With a Sony digital camera, pictures of representative samples were captured. Using a linear eye piece micrometer, the lengths of the long were measured.

Histological examination

Rat pups' femurs were meticulously dissected to remove any soft tissue before being examined under a light microscope. Immediately after, the distal portion of every bone, including the diaphysis and distal metaphysis, was fixed for two days in 10% neutral buffered formaldehyde. Following fixation, EDTA (5.5 g EDTA in 90 ml distilled water and 10 ml formaldehyde (37–40%)) was used to prepare the decalcified bones. The solution was changed daily for the duration of the three-week decalcification process[39]. Paraffin blocks were created by drying and processing the decalcified specimens. Coronal slices, measuring 6 µm in thickness and aligned with the femur's long axis, were cut and stained with either hematoxylin and eosin[40] or alizarin red S[41]. Histological slides were examined under a microscope and photomicrographed with an Olympus BX41 microscope.

Chromosomal preparation and mitotic index assay

Chromosomal preparation was performed according to Anwar *et al*.[42]. Adult rats and pups from each group were injected with colchicine at a dose of 4 mg/kg B.wt, and then the rats and pups were separated and sacrificed two hours later after injection. The cell suspension of bone marrow was centrifuged on 1000 rpm for 5 min, and supernatant was subsequently removed. Cells were then incubated in hypotonic solution at 37˚C for 30-60 min., followed by fixation, which was repeated three times. Dripping method was used to prepare the cells on clean slides, which were left to dry at RT. The slides were then stained using 5% Giemsa staining buffer. For each animal, 400 metaphase cells were examined and photographed under light microscope (400X Olympus BX 41, Japan), to demonstrate different types of malformations.

The mitotic index was estimated in 400 cells for each group according to the following formula:

Mitotic index (MI) = (Number of dividing cells/ Total examined cells) x100

Data evaluation & statistical analysis

Data were displayed as mean±(SD). Using GraphPad Prism, version 9, one-way ANOVA and Tukey's post-hoc test for multiple comparisons were used for the statistical analysis of the data. When $P>0.05$, differences were deemed not significant. Based on the derived *P values*, the significance of the data was categorized into two groups: high significance $P<0.001$ and low significance $P\leq0.05$.

RESULTS

Morphometric parameters

Morphology of uteri

(Figures 1 A,B) shows that the uteri of the pregnant rats in the control and propolis groups had a healthy, bright appearance, a proper distribution of the implanted fetuses between the two horns, and normal fetal growth, without any absorption sites. In contrast, pregnant rats administered methomyl illustrated a decrease in the number of implantation sites and the resorption of the great number of fetuses (Figures 2 C,D). Meanwhile, the uteri of pregnant dams that received propolis and methomyl in combination displayed a decrease in the number of resorption sites (Figure 2E).

Resorptions, resorption rate percentage and fetal viability percentage

(Table 1, Figures 1,2) illustrate a significant increase in the resorption rate in the methomyl group compared with control and propolis groups (57.69%). Contrarily, the combined group indicated a significant decrease in resorption rate compared with the methomyl group as a result of propolis administration (7.69%). Furthermore, fetal viability did not change significantly between the methomyl group and the control group.

Fetal weight

(Table 1, Figures 2,3) indicate that the fetal body weights of fetuses in both the groups of control & propolis were nearly similar (5.6±0.51 and 5.8±0.72 g, respectively) with nonsignificant difference. However, there was a significant decrease ($P<0.001$) in the fetal body weight in the methomyl group compared with the control group (2.9±0.39 g). Moreover, the combined group showed amelioration in body weight (4.2±0.76 g) with a significant increase in a case of comparison with the methomyl group and significant decrease ($P=.013$) in a case of comparison with the control one.

Fetal crown-rump length

As seen in (Table 1, Figures 2,3), there was an insignificant difference in the fetal length between the control and propolis groups (5.2±0.54 and 5.7±1.05 cm, respectively). Administration of methomyl give a significant decrease ($P=0.002$) in the crown-rump length of the fetuses compared with the control group (3.2±0.53). Combined group displayed a nonsignificant increase compared in a case of comparison with methomyl (3.7±0.50) and significant decrease ($P=0.02$) in a case of comparison with the control.

Placental weight and Feto-Placental ratio

(Table 1) illustrates an insignificant difference in the placental weight between the control and propolis groups (0.69±0.08 and 0.73±0.03 g, respectively), similar to the feto-placental ratio (8.1 and 7.9, respectively). Also, the methomyl group decrease significantly (P<.001) in placental weight (0.42±0.01 g) and the feto-placental ratio (6.9) in compared with the control. It is noteworthy to mention that administration of methomyl with propolis significantly increased (P=0.002) the placental weight (0.57±0.08) and the feto-placental ratio (7.4) in compared with methomyl and significantly decreased (P=0.02) them compared with the control.

Morphological abnormalities

The morphology of rat fetuses aged 20 days was investigated thoroughly. Our results showed that the control and propolis fetuses generally displayed normal morphology without any morphologic abnormalities (Figures 2 A,B). Administration of methomyl, however, induced intrauterine growth restriction compared with the control group. Moreover, the fetuses displayed many morphological abnormalities (Figures 2 C-G). The skin, jaws, trunk, and ear pinna of this group had a high frequency of abnormalities (Table 2). Umbilical hernia was present in 54.54% of fetuses (Figures 2 C,F). In 45.45% of fetuses, soft membranous skin was found (Figures 2 D-F). Subcutaneous hemorrhage was detected in 22.73% of fetuses in the methomyl group (Figures 2 E-F). Clear deviation from normal curvatures (kyphosis) was found in the trunk area of 13.64% of fetuses (Figure 2G). Hypognathia (9.09%) and small ear pinna (27.27%) were obvious head abnormalities (Figures 2 D,F,G). When administered after methomyl, propolis extract significantly reduced the morphological alterations caused by methomyl. The shape, length, and weight of the fetuses in this group significantly improved (Table 2, Figure 2H), with no apparent morphological abnormalities.

Endoskeletal investigation

The 20-day-old rat fetuses' double-stained endoskeleton in the control and propolis groups displayed a high degree of ossification in the skull including per-maxilla, mandible, nasal, frontal, parietal, interparietal and squamosal, which were all stained heavily red. In addition, the vertebral column, sternum and ribs showed a high degree of ossification. Additionally, most of the long bones of the fore and hind limbs were completely ossified, except for the phalanges which were partially ossified (Figures 3 A,B).

In contrast, fetuses maternally treated with 2 mg/kg methomyl showed a high percentage of ill-ossification in most of the endoskeleton, including the long bones, as well as the skull (3C). Moreover, the combined group showed improvements in the ossification of all bones as a result of propolis administration (Figure 3D).

(Table 3) demonstrates the measurements of the ossification center of long bones. The length of the ossified center of the humerus, radius and ulna in the control and propolis groups showed nonsignificant difference (0.82±0.04 cm and 0.88±0.06 cm (Humerus), (0.58±0.06 cm and 0.60±0.04 cm (Radius), (0.46±0.04 cm and 0.53±0.02 cm (ulna), for the two groups respectively). Contrarily, the length of the humerus, radius and ulna decreased significantly (P<0.001) in fetuses in the methomyl group compared with the control group (0.30±0.19 cm, 0.14±0.09 cm, 0.10±0.07 cm, for thtree bones respectively). Meanwhile, administration of propolis with methomyl significantly increased the length of the humerus (P=0.009), radius (P=0.009) and ulna (P=0.001) compared with the methomyl group (0.56±0.09 cm, 0.28±0.02 cm, 0.22±0.02 cm, for the three bones respectively).

Similarly, the length of the femur, tibia and fibula in the control and propolis groups showed nonsignificant differences (0.43±0.02 cm, 0.52±0.02 cm, 0.36±0.02 cm (control group) and 0.46±0.02 cm, 0.57±0.01 cm, 0.41±0.02 cm (propolis group), for the three bones respectively). There was a significant decrease in the length of the femur (P<0.001), tibia (P=0.001) and fibula (P<0.001) in fetuses maternally administered methomyl (0.19±0.12 cm, 0.26±0.17 cm and 0.14±0.13 cm, for the three bones respectively). The combined group showed significant increase in the length of femur (0.31±0.04 cm, P=0.01), tibia (0.47±0.05 cm, P=0.008) and fibula (0.32±0.05 cm, P=0.003) compared with that of the methomyl group and nonsignificant difference compared with the control group.

Histological investigation

Hematoxyline and eosin (H&E) stainining

(Figures 4 A,B) shows that the femur of the rat pups aged 14 days in the groups of control and propolis displayed a distal epiphyseal plate with four distinct layers, namely the resting layer, proliferative layer, noticeable hypertrophic layer and calcification layer with regular chondrocyte column arrangement. The diaphysis cortical bone demonstrated a number of haversian systems with prominent haversian canals and osteocytes in their lacunae. On the other hand, the distal epiphyseal plate in the femur of the 14-day-old rat pups in the methomyl group illustrated some histological changes including disordered chondrocyte column arrangement, and the chondrocytes seemed smaller in size and atrophied (Figures 4 C,F). In addition, the presence of many acidophilic osteoclasts was evident (Figure 4F). Moreover, congested blood vessels appeared between the osteocytes (Figure 4E). The haversian canals between lacunae had a widened appearance (Figures 4 D,G). Propolis administration resulted in amelioration of the structure of the femur of the rat pups aged 14 days in the combined group (Figures 4H).

Alizarin red stain

The alizarin red stained sections of the femur of the the rat pups aged 14 days in the groups of control and propolis revealed insignificant differences in the thickness

of the shaft cortex (0.91±0.042 and 0.93±0.020 mm, respectively). On the other hand, the femur of methomyl displayed decrease (P<0.001) in the thickness of the shaft cortex (0.46±0.015 mm) compared with the control group. Meanwhile, the thickness of the shaft cortex of the femur in the combined group displayed a significant increase (P<0.001) compared with that in the methomyl group (0.75±0.023 mm) (Table 4, Figure 5).

Molecular investigations

Chromosomal aberrations

(Table 5, Figure 6) show that the bone marrow cells in the rat pups aged 14 days and mothers in the groups of control and propolis displayed normal number of chromosomes (42 chromosomes) with a small percentage of chromosomal abnormalities, accounting for 13% (control) and 7.5% (propolis) in pups and 10% (control), and 6.75% (propolis) in their dams. The current results showed that methomyl administration induced both structural and numerical chromosomal abnormalities in the bone marrow cells of rat pups and mothers. Comparing with the control, the occurance of structural and numerical chromosomal abnormalities was considerably higher in the methomyl group, where it was 53.75% in pups and 44.75% in mothers. The numerical chromosomal aberrations in the methomyl group included 4.75% hypoploid and 6.5% hyperploid patterns in rat pups, while there were 3.25% hypoploid and 1% hyperploid patterns in the mothers. The structural chromosomal abnormalities in rat pups and mothers included fragmentation (F) (17.25%, and 14.5%, respectively), ring chromosome (R) (7.75%, and 9.25%, respectively), centric attenuation (Ca) (5%, and 6%, respectively), centric fusion (Cf) (6.75%, and 7%, respectively), and end to end association (Ee) (5.75%, and 3.5%, respectively). Interestingly, after propolis administration, the numerical and structural chromosomal aberrations decreased in rat pups and mothers (18.25%, and 14.75 % , respectively).

Mitotic index

The mitotic index was calculated and expressed as a percentage to assess the impact of methomyl administration on cellular proliferation. As seen in (Table 6), the control and propolis groups showed nonsignificant differences in the percentage of mitotic division in rat pups (23.8±3.3 and 24.40±1.5, for the control and propolis groups, respectively) and mothers (23.2±3.1 and 23.6±2.4, for control and propolis groups, respectively). On the other hand, methomyl administration showed a significant decrease (P<0.001) in the percentage of dividing cells compared with the control group in rat pups and mothers (8.6±1.1, and 10.6±1.3, respectively). As the result of propolis administration, the mitotic index displayed significant increase (P<0.001) in both rat pups and mothers (18.2±3.5, and 18.4±3.05, respectively) compared with that of the methomyl group and significant decrease (P=0.02, and P=0.04, for pups and mothers respectively) compared with the control group.

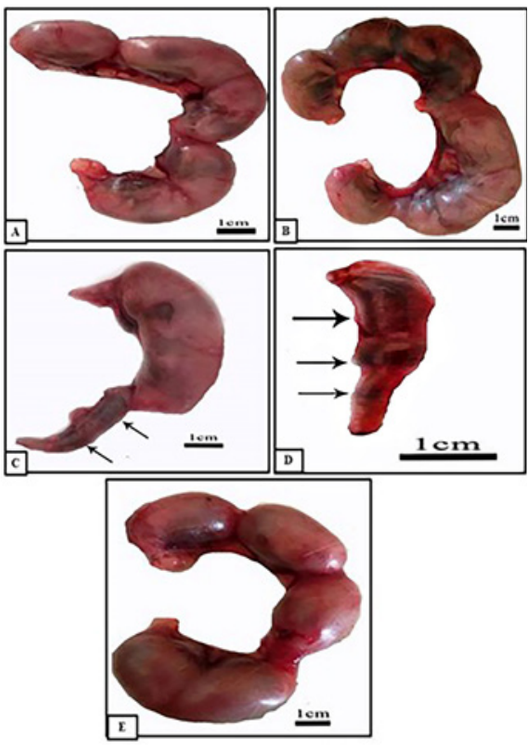

Fig. 1: Representative photographs of the morphology of uteri from the control (A), propolis (B), methomyl (C&D) and combined (E) groups showing a normal appearance of uteri (A&B), absorbed sites in the uteri (arrows) (C&D), and the uteri of the combined group showing no absorption sites (E).

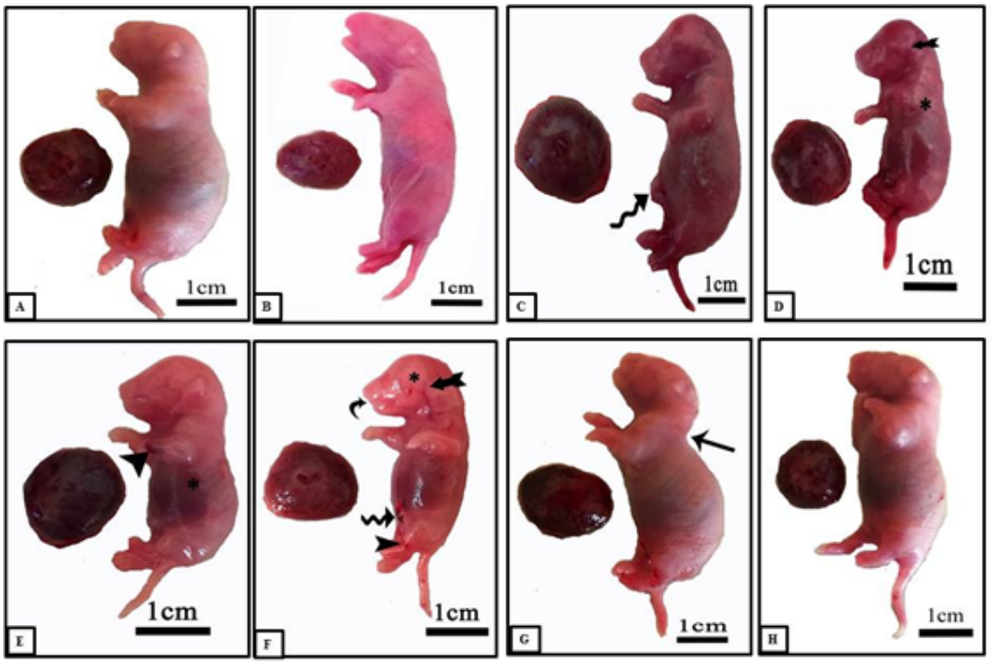

Fig. 2: Representative photographs of the morphological malformations in rat fetuses aged 20 day in varios groups. A Control, B propolis, C-G methomyl and H combined groups. (A&B) Normal morphology of the fetuses. (C-G) Umbilical hernia (wavy arrow), small ear pinna (tailed arrow), soft membranous skin (*), subcutaneous hemorrhage (arrow head), hypognatha (currved arrow) and kyphosis (arrow). (H) Improved morphology of the fetuse as a result of maternal propolis administration.

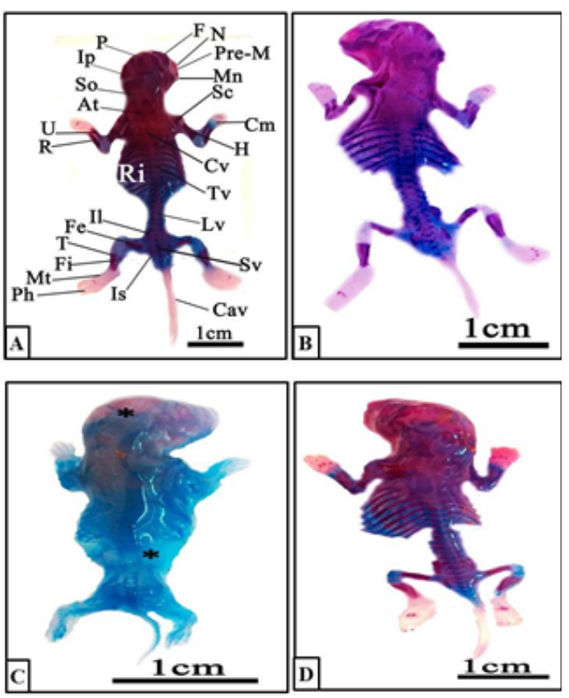

Fig. 3: Representative photographs of dorsal views of the endoskeletal system of rat-fetuses aged 20 days. The control A, propolis B, methomyl C and combined D groups double stained with Alizarin red S and Alcian blue showing a well-formed endoskeleton with dense ossification of the skull, vertebral column, ribs, fore limb and hind limb (A&B), ill-ossified endoskeletal elements (C *) and improved ossification in endoskeleton (D).

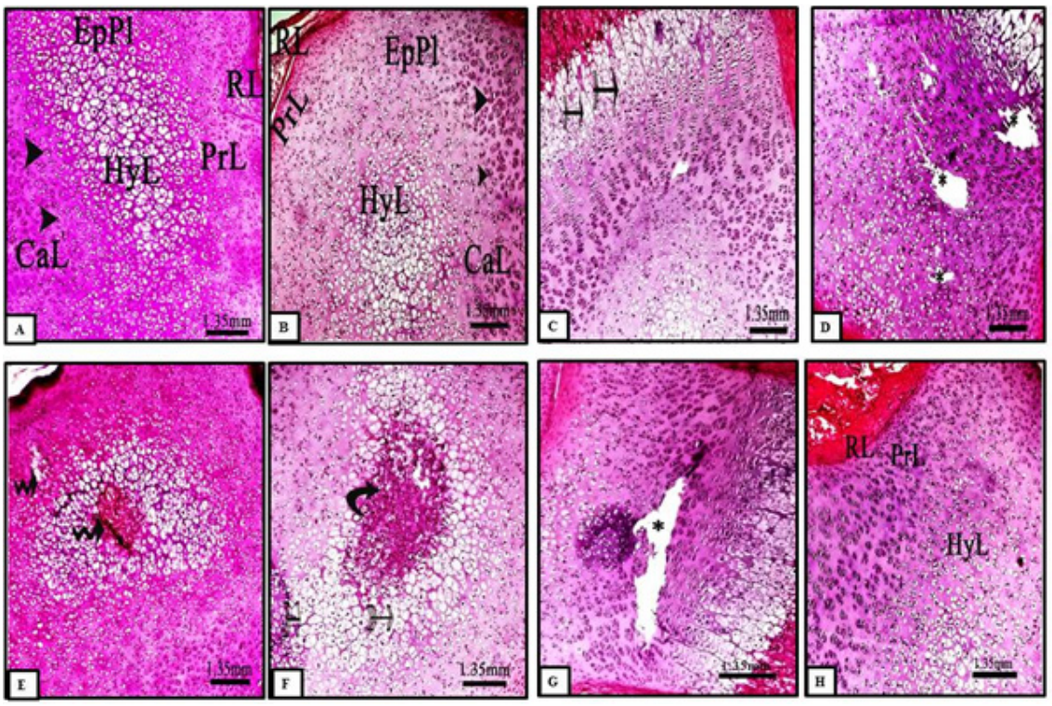

Fig. 4: Representative H&E stained sections in the femur of the rat pups aged 14 days in the groups of control and propolis A, B, methomyl C-G and methomyl+propolis H groups showing the epiphyseal plate layer (EpPl), resting layer (RL), proliferative (PrL) layer, hypertrophic layer (HyL), calcification layer (CaL), osteocytes (head arrow) (A&B), atrophied and disordered chondrocyte column arrangement (tailed arrow) (C&F), widened haversian canals (*) (D&G), congested blood vessels (wavy arrow), and acidophilic osteoclasts (curved arrow) (F). Amelioration in the epiphysal plate of the femur in the methomyl+propolis group (H).

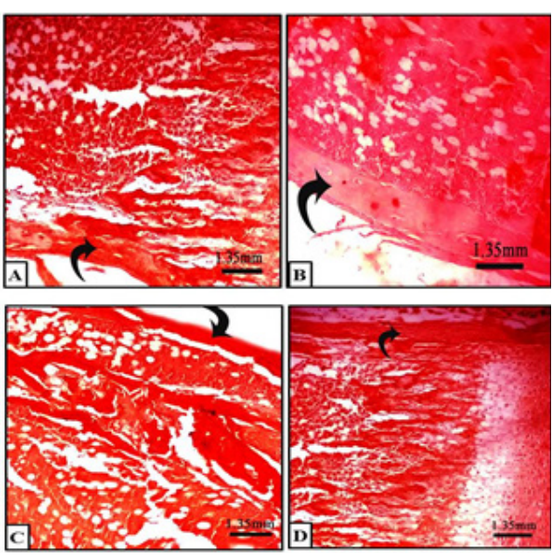

Fig. 5: Representative Alizarin red stained sections in the femur of the rat pups aged 14 days in the groups of control A, propolis B, methomyl C and methomyl+propolis D groups showing the cortical layer (curved arrow).

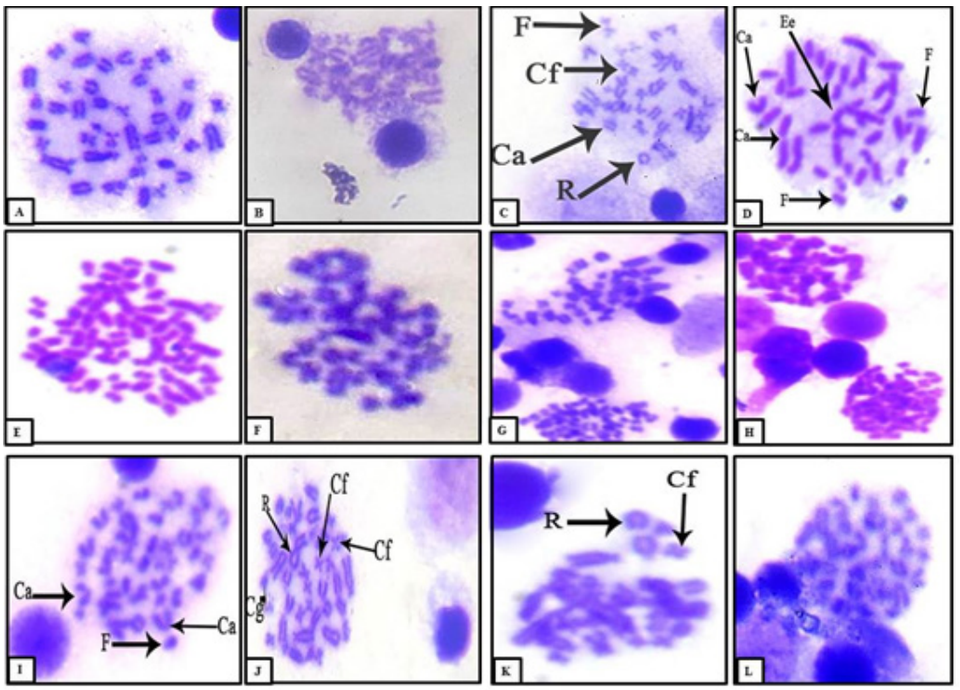

Fig. 6: Representative photomicrographs of bone marrow cells of in the rat pups aged 14 days (A-F) also rat mothers (G-L) from control (A&G), propolis (B&H), methomyl (C-E&I-K) and combined (F&L) groups showing different chromosomal abnormalities; normal number of chromosomes (42 chromosomes). Fragment (F), centric fusion (Cf), centric attenuation (Ca), ring chromosome (R), end to end association (Ee), hyperploidy (E), ring chromosome (R), centric gab (Cg).

Table 1: Morphometric analysis in rat fetuses aged 20 day in various groups. n= 52 where (n) is the number of embryos in each group.

Groups	Resorptions (count)	Resorption rate (%)	Fetal weight (g)	Fetal length (cm)	Placenta weight (g)	Feto-Placental ratio
Control	0	0%	5.6±0.51	5.2±0.54	0.69±0.08	8.1
Propolis	0	0%	5.8±0.72	5.7±1.05	0.73±0.03	7.9
Methomyl	30	57.69%	2.9±0.39**	3.2±0.53*	0.42±0.01**	6.9
Combined	4	7.69%	4.2±0.76*#	3.7±0.50*	0.57±0.08*#	7.4

(* &**) low and high significance respectively related to control.
(# &##) low and high significance respectively related to methomyl. n=6.

Table 2: Fetal morphological abnormalities (%) of rat fetuses aged 20 day in various groups.

Groups	Umbilical hernia (%)	Soft membranous skin (%)	Subcutaneous hemorrhage (%)	Kyphosis (%)	Hypognathia (%)	Small ear pinna (%)
Control (52)	7.69% (4)	9.62% (5)	3.85% (2)	0% (0)	0% (0)	3.85% (2)
Propolis (52)	5.77% (3)	7.69% (4)	1.92% (1)	0% (0)	0% (0)	3.85% (2)
Methomyl (22)	(12) 54.54%	45.45% (10)	22.73% (5)	13.64% (3)	9.09% (2)	27.27% (6)
Combined (48)	(9) 18.75%	14.58% (7)	6.25% (3)	4.17% (2)	0% (0)	8.33% (4)

Table 3: Length of ossified long bones in rat fetuses aged 20 day in various groups.

Groups	Humerus	Radius	Ulna	Femur	Tibia	Fibula
Control	0.82±0.04	0.58±0.06	0.46±0.04	0.43±0.02	0.52±0.02	0.36±0.02
Propolis	0.88±0.06	0.60±0.04	0.53±0.02	0.46±0.02	0.57±0.01	0.41±0.02
Methomyl	**0.30±0.19	0.14±0.09**	0.10±0.07**	0.19±0.12**	0.26±0.17*	0.14±0.13**
Combined	#*0.56±0.09	0.28±0.02**#	0.22±0.02**#	0.34±0.04#	0.47±0.05#	0.32±0.05#

(* &**) low and high significance respectively related to control.
(# &##) low and high significance respectively related to methomyl. n=6.

Table 4: Thickness of the shaft cortex in the femur of rat pups aged 14 days in different groups.

Groups	Thickness (mm)
Control	0.91±0.042
Propolis	0.93±0.020
Methomyl	0.46±0.015**
Combined	0.75±0.023**##

(* &**) low and high significance respectively related to control.
(# &##) low and high significance respectively related to methomyl. n=6.

Table 5: Chromosomal aberrations induced in bone marrow cells of in the rat pups aged 14 days and their dams in different groups.

Groups Aberration	Control		Propolis		Methomyl		Combined	
	Pups	Dams	Pups	Dams	Pups	Dams	Pups	Dams
Fragmentation	2.25% (9)	3.5% (14)	0.75% (3)	2.75% (11)	17.25% (69)	14.5% (58)	4.25% (17)	5.5% (22)
Ring chromosome	2% (8)	1% (4)	1% (4)	0	7.75% (31)	9.25% (37)	5% (20)	2.25% (9)
Centric attenuation	3.5% (14)	2% (8)	1.75% (7)	1% (4)	5% (20)	6% (24)	2.5% (10)	1.5% (6)
Centric fusion	2.75% (11)	1.25% (5)	2.5% (10)	2.25% (9)	6.75% (27)	7% (28)	3% (12)	4.5% (18)
End to end association	1% (4)	0	0	0	5.75% (23)	3.5% (14)	2% (8)	0.5% (2)
Hypoploidy	0.5% (2)	2.25% (9)	0	0.75% (3)	4.75% (19)	3.25% (13)	0	0.5% (2)
Hyperploidy	1% (4)	0	1.5% (6)	0	6.5% (26)	1% (4)	1.5% (6)	0
Total	13% (52)	10% (40)	7.5% (30)	6.75% (27)	53.75% (215)	44.75% (179)	18.25% (73)	14.75 % (59)

The number of examined cells in each group= 400.

Table 6: Mitotic index % in bone marrow cells in in the rat pups aged 14 days and mothers in various groups.

Groups	Pups	Mothers
Control	23.8±3.3	23.2±3.1
Propolis	24.40±1.5	23.6±2.4
Methomyl	8.6±1.1**	10.6±1.3**
Combined	18.2±3.5*##	18.4±3.05*#

(* &**) low and high significance respectively related to control.
(# &##) low and high significance respectively related to methomyl. n=6.
The number of examined cells in each group= 400.

DISCUSSION

Our study showed that methomyl administration resulted in profound morphological defects, both in the uterine horns and the fetuses, as evidenced by an increased resorption rate, decreased fetal body weight and crown-rump length, decreased placental weight and placental index and increased fetal morphological abnormalities. Similarly, the study of Abou Zeid[7] reported that insecticides introduced developmental and morphological toxic effects. Similarly, some studies found that chronic exposure to methomyl had a direct toxic effect on the female reproductive system, where the authors reported that methomyl affected the implantation sites, fertility index and fetal weights in newborns of pregnant rats[10,11]. In the same context, He *et al*.[43] and Toledo *et al*.[44] reported that methomyl decreased the weight of the mothers and their fetuses, as well as the number of implantation sites, live fetuses and increased the incidence of dead embryos and resorptions.

The effect of methomyl could be due to decreased food intake through alterations in hormonal balance or the cytotoxic effect of methomyl[45]. Moreover, this could be because pesticides have the power to go over the barrier of placental and travel into the fetal bloodstream. Thus, pesticide exposure could be related to an increased risk of having fetuses with some sort of structural impairment and increased number of absorption sites[46].

Additionally, our study indicated the ameliorative role of propolis on morphometric parameters in rat fetuses. Similarly, Usman *et al*.[36] found that propolis (300 mg/kg) improved implantation losses and placental oxidative stress markers such as malonaldehyde and protein carbonyl, in addition to increased maternal weight gain and fetal body weight. Moreover, it has been reported that different doses of Brazilian propolis improved body weights in rats[47,48]. This may be due to the antioxidant effect of flavonoids and phenols found in propolis[49].

The ossification of the skeletal system in rat fetuses and pups in the methomyl group was reduced and the histological structure of the bone was evidently altered. These results are in agreement with other previous

studies that reported that rat fetuses maternally exposed to methomyl at the same dose had a significant increase in the percentage of morphological, skeletal and visceral abnormalities[7,50]. The decreased ossification of the fetal skeletal system may be due to decreased fetal body weight. A decline in the supply of calcium and magnesium ions to growing fetuses leads to an interruption in the development of bones, which may be related to the effects of pesticides on calcium metabolism[51].

Interestingly, the current study indicated that propolis administration ameliorated the ossification of the fetal skeleton and histological composition of bone. A study by Dillasamola *et al*.[33] indicated that administration of propolis in lactating mothers increased fetuses' body weight and repaired skeletal defects caused by caffeine induction. The fish liver oil, and propolis with antiepileptic drugs has been reported to improve the histo-pathological defects and morphometric parameters in bone associated with antiepileptic drug that make osteoporosis[52], possibly by increasing the number of osteocytes, increasing the thickness of the shaft cortical width, accelerating osteogenesis and bone formation, and shortening the consolidation phase[53,54].

The current work showed that methomyl administration induced a number of chromosomal abnormalities. In addition, methomyl administration decreased the mitotic index in bone marrow cells in both rat pups and mothers. These results are in agreement with Zedin and Galal[13] who found that malathion insecticide induced chromosomal damage in bone marrow cells of male albino rats. This might be because insecticides contain clastogenic compounds, which are capable of inducing chromosomal abnormalities[55]. In contrast, the combined group displayed a decrease in the number of chromosomal aberrations and an increase in the mitotic index in bone marrow cells in rat pups and mothers as a result of propolis administration. Another study indicated that the administration of honey improved the frequency of chromosomal aberrations in rats[34]. This may be due to the high efficiency of propolis as an antioxidant where it scavenges the produced free radicals and hence decreases DNA damage and chromosomal abnormalities[56].

CONCLUSIONS

The current work illustrated that maternal exposure to methomyl during the period of pregnancy and lactation had harmful effects on morphology and endoskeletal strcture, and induced chromosomal aberations during fetal and postnatal development. Intersetingly, the present study demonstrated that propolis had an ameliorative effect on the developing fetuses and pups against the deleterious effects of methomyl. Hence, our study recommends that it is important to restrict the usage of methomyl as an insecticide and minimize its exposure, especially during pregnancy and lactation. Additionally, we recommend that propolis could be taken as a dietary supplement in pregnant or lactating mothers. Consequently, it is essential that pregnant women and nursing mothers understand the possible dangers of exposure to methomyl and evaluate the benefits of consuming propolis as a dietary supplement.

AUTHOR CONTRIBUTIONS

All authors have conceived and planned the experiments. AM, AF Methodology, investigation, formal analysis, visualization, writing – original draft. EH formal analysis, resources, writing – reviewing and editing. BG conceptualization, supervision, project administration, resources, writing – review and editing. All authors discussed the results and commented on the manuscript. All authors have read and approved the manuscript.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Abouzaid O, Abd El-Mageid A, Alsadek E: Protective and treatment effect of garlic extract on biochemical changes induced by pesticides in rats. BVMJ (2015) 29:205-213. Doi 10.21608/BVMJ.2015.31820
2. Choudri B, Charabi Y: Pesticides and herbicides. Water Environ Res (2019) 91:1342-1349. https://doi.org/10.1002/wer.1380
3. Tago D, Andersson H, Treich N: Pesticides and health: a review of evidence on health effects, valuation of risks, and benefit-cost analysis. Adv Health Econ Health Serv Res (2014) 24:203-295
4. Vale A, Lotti M: Organophosphorus and carbamate insecticide poisoning. Handb Clin Neurol (2015) 131:149-168. doi: 10.1016/B978-0-444-62627-1.00010-X
5. Akashe M, Pawade U, Nikam A: Classification of pesticides: a review. Int J Res (2018) 9:144-150. DOI: 10.7897/2277-4343.094131
6. Silberman J, Taylor A (2022): Carbamate toxicity. Bookshelf ID: NBK482183PMID: 29489157
7. Abou Zeid S: Developmental toxicity of acetamiprid in rats. J Pharm Pharm Sci (2018) 7:113-126. DOI: 10.20959/wjpps20182-10933
8. Mansour S, Mossa A, Heikal T: Effects of methomyl on lipid peroxidation and antioxidant enzymes in rat erythrocytes: In *vitro* studies. Toxicol Ind Health (2009) 25:557-563. doi: 10.1177/0748233709349829
9. Shalaby M, El Zorba H, Ziada R: Reproductive toxicity of methomyl insecticide in male rats and protective effect of folic acid. Food Chem Toxicol (2010) 48:3221-3226. doi: 10.1016/j.fct.2010.08.027
10. Mokhtar H, Abdel-Latif H, ElMazoudy R, Abdelwahab W, Saad M: Effect of methomyl on fertility, embryotoxicity and physiological parameters in female rats. J of Appl Pharm Sci (2013) 3:109-119. DOI: 10.7324/JAPS.2013.31220

11. Rachid M, Ibrahim Y: Vitamin E protects against methomyl-induced reproductive toxicity in pregnant female rats. Endocrine Abstracts (2013) 32:P501. DOI: 10.1530/endoabs.32.P501

12. Rachid M, Mokrani N , Mosbahi X , Rouabhi S, Mantovani A: Effect of methomyl on the biochemical and reproductive parameters in pregnancy rats: the protective role of Pistacia Lentiscus oil. Endocrine abstracts (2016) 41: EP671 | DOI: 10.1530/endoabs.41.EP671

13. Zedin N, Galal O: Evaluation of hematological, cytogenetical and biochemical effects of malathion and spinosad on male albino rats. Amer-Euras J Toxocol Sci (2012) 4:118-130. DOI: 10.5829/idosi.aejts.2012.4.2.64200

14. Atallah M, Badawy G, Abdallah F, El-Borm H: Assessment of methomyl-induced adrenal gland disruption in rat fetuses and pups: Potential protective effects of propolis supplementation. JEZ-A (2023), 10.1002/jez.2777. https://doi.org/10.1002/jez.2777

15. Beutler J: Natural products as a foundation for drug discovery. Curr Protoc Pharmacol (2009) 46:1-9. doi: 10.1002/0471141755.ph0911s46

16. Luo X, Dong Y, Gu C, Zhang X, Ma H: Processing technologies for bee products: an overview of recent developments and perspective. Front Nutr (2021) 8:1-30. https://doi.org/10.3389/fnut.2021.727181

17. Oryan A, Alemzadeh E, Moshiri A: Potential role of propolis in wound healing: biological properties and therapeutic activities. Biomed Pharmacother (2018) 98:469-483. doi: 10.1007/s00403-023-02789-x

18. Kleczek K, Wilkiewicz-Wawro E, Wawro K, Makowski W, Murawska D, Wawro M: The effect of dietary propolis supplementation on the growth performance of broiler chickens. Pol J Nat Sci (2014) 29:105-117.

19. Huang S, Zhang C, Wang K, Li G, Hu F: Recent advances in the chemical composition of propolis. Molecules (2014) 19:19610-1932. DOI: 10.3390/molecules191219610

20. Veiga F, Gadelha M, da Silva M, Costa M, Kischkel B, de Castro-Hoshino L, Sato F, Baesso M, Voidaleski M, Vasconcellos-Pontello V, Vicente V, Bruschi M, Negri M, Svidzinski T: Propolis Extract for Onychomycosis Topical Treatment: From Bench to Clinic. Front Microbiol (2018) 9:779. doi: 10.3389/fmicb.2018.00779.

21. Silva H, Francisco R, Saraiva A, Francisco S, Carrascosa C, Raposo A: The cardiovascular therapeutic potential of propolis-a comprehensive review. Biology (2021) 10:1-20. doi: 10.3390/biology10010027

22. Kischkel B, Castilho P, Oliveira K, Rezende P, Bruschi M, Svidzinski, Negri M: Silver nanoparticles stabilized with propolis show reduced toxicity and potential activity against fungal infections. Future Microbiol (2020) 15:521-539. doi: 10.2217/fmb-2019-0173

23. Soylu E, Ozdemir A, Erturk E, Sahinler N, Soylu S: Chemical composition and antifungal activity of propolis against Penicillium digitatum. Asi J Chem (2008) 20:4823-4830

24. Aygun A, Sert D: Effects of prestorage application of propolis and storage time on eggshell microbial activity, hatchability, and chick performance in Japanese quail (Coturnix coturnix japonica) eggs. Poult Sci J (2013) 92:3330-3337. doi: 10.3382/ps.2013-03291.

25. Magnavacca A, Sangiovanni E, Racagni G, Dell'Agli M: The antiviral and immunomodulatory activities of propolis: An update and future perspectives for respiratory diseases. Med Res Rev (2022) 42:897-945. doi: 10.1002/med.21866

26. Gregoris E, Fabris S, Bertelle M, Grassato L, Stevanato R: Propolis as potential cosmeceutical sunscreen agent for its combined photoprotective and antioxidant properties. Int J Pharma (2011) 405:97-101. doi: 10.1016/j.ijpharm.2010.11.052.

27. Mahmoud M, Abdel-Mohsein H, Farghali M: Antioxidant properties of chinese propolis in ross broilers exposed to heat stress in Egypt. Open J Vet Med (2015) 5:197-209. DOI: 10.4236/ojvm.2015.59027

28. Kalsum N, Sulaeman A, Setiawan B, Wibawan I: The effect of liquid Trigona spp. propolis of the immune system of Sprague Dawley rats infected Sthapylococcus aureus. J Gizi dan Pangan (2017) 12:23-30. DOI: 10.25182/jgp.2017.12.1.23-30

29. Oršolić N, Terzić S, Šver L, Bašić I: Polyphenolic compounds from propolis modulate immune responses and increase host resistance to tumour cells. Food Agr Immunol (2005) 16:165-179. https://doi.org/10.1080/09540100500258484

30. Copur G, Camci O, Sahinler N, Gul A: The effect of propolis eggshell coatings on interior egg quality. Archiv für Geflügelkunde (2008) 72:35-40

31. Akpinar G, Canogullari S, Baylan M, Alasahan S, Aygun A: The use of propolis extract for the storage of quail eggs. J App Poult Res (2015) 24:427-435. https://doi.org/10.3382/japr/pfv043

32. Chiu H, Han Y, Shen Y, Golovinskaia O, Venkatakrishnan K, Wang C: Chemopreventive and chemotherapeutic effect of propolis and its constituents: a mini-review. J Cancer Prev (2020) 25:70-78. doi: 10.15430/JCP.2020.25.2.70

33. Dillasamola D, Almahdy A, Sari N, Putri B, Diliarosta N: Evaluation of Propolis and Milk Administration on Caffein-Induced Mus musculus fetus skeletal. Pharm Sci Res (2018) 5:1

34. Shahrour A, Zowail M, Sharafeldin K: The protective role of honey against cytotoxicity of cadmium chloride in mice. Afr J Biotechnol (2016) 15:2620-2626. DOI: 10.5897/AJB2016.15426

35. Christen S, Hill T, Williams M: Effects of tempered barely on milk yield, intake and digestion kinetic of lactating Holstein cows. J Dairy Sci (1996) 79:1394-1399. doi: 10.3168/jds.S0022-0302(96)76497-4.

36. Usman U, Bakar A, Mohamed M: Propolis improves pregnancy outcomes and placental oxidative stress status in streptozotocin-induced diabetic rats. BMC Complement Altern Med (2018) 18:324. https://doi.org/10.1186/s12906-018-2391-6

37. Cortés-Delgado N, Perez-Torres J, Hoyos J: Staining procedure of cartilage and skeleton in adult bats and rodents. Int J Morphol (2009) 27:1163-1167. doi: 10.3109/10520298109067325

38. Badawy G, Sakr S, Atallah M: Comparative study of the skeletogenesis of limb autopods in the developing chick Gallus domesticus and Toad Bufo regularis. RJPBCS (2012) 3:966-988

39. Bancroft J, Gamble M (2008): Theory and practice of histological techniques. 6th ed. London, NewYork, Philadelphia: Churchill Livingstone

40. Humason G (1979): Animal tissue techniques. 4th edn, Freeman, W.Co., San Francisisco

41. Hosoya A, Hoshi K, Sahara N, Ninomiya T, Akahane S, Kawamoto T, Ozawa H: Effects of fixation and decalcification on the immunohistochemical localization of bone matrix proteins in fresh-frozen bone sections. Histochem Cell Biol (2005) 123:639-646. doi: 10.1007/s00418-005-0791-4

42. Anwar W, Khalil M, Wild C: Micronuclei, chromosomal aberrations and aflatoxinalbumin adducts in experimental animals afterexposure to aflatoxin B. Mutation Research (1994) 322:61-67. https://doi.org/10.1016/0165-1218(94)90033-7

43. He D, Han G, Zhang X, Sun J, Xu Y, Jin Q, Gao Q: Oxidative stress induced by methomyl exposure reduces the quality of early embryo development in mice. Zygot (2022)30:57-64. doi: 10.1017/S0967199421000277

44. Toledo J, Gamalo L, Suetos K, Galapon M, Maligalig M: Estrous cycle and early pregnancy of white mice exposed to methomyl. Pollution (2019) 5:279-286. Doi 10.22059/POLL.2018.265744.518

45. Al-Shinnawy M: Vitamin "C" as ameliorative agent against thiodicarb toxicated male albino rats (rattus norvegicus). Egypt Acad J biol Sci (2008) 1:177-187. Doi 10.21608/EAJBSA.2008.15749

46. Costa-Maldonado B, Sanchez-Ramirez S. Reza-Lopez B, Carrillo M: Effects of exposure to pesticides during pregnancy on placental maturity and weight of newborns: A cross-sectional pilot study in women from the Chihuahua State, Mexico. Hum Exp Toxicol (2009) 28:451-459. doi: 10.1177/0960327109107045

47. Abo-Salem O, El-Edel R, Harisa G, El-Halawany N, Ghonaim M: Experimental diabetic nephropathy can be prevented by propolis: effect on metabolic disturbances and renal oxidative parameters. Pak J Pharm Sci (2009) 22:205-210

48. Arabameri A, Sameni H, Bandegi A: The effects of propolis extract on ovarian tissue and oxidative stress in rats with maternal separation stress. Int J Reprod Bio Med (2017) 15:509-520

49. Usman U, Bakar A, Mohamed M: Phytochemical screening and comparison of antioxidant activity of water and ethanol extract propolis from Malaysia. Int J Pharm Pharm Sci (2016) 8:413-415

50. Alharbi F: Effect of methomyl on fetal development in female rats. Egypt J Chem Environ Health (2018) 4:32- 42. doi 10.21608/EJCEH.2018.241063

51. Farias P, Marcelino G, Santana L, de Almeida E, Guimarães R, Pott A, Hiane P, Freitas K: Minerals in Pregnancy and Their Impact on Child Growth and Development. Molecules (2020) 25(23):5630. doi: 10.3390/molecules25235630. doi: 10.3390/molecules25235630

52. Morsy F, Gamal El Din A, Farrag A, Shaffie N, Badawi M, Sharaf W: Protective effect of fish liver oil and propolis on anticonvulsant drugs-induced osteoporosis. JASMR (2014) 9:81-89. doi: 10.1016/j.eplepsyres.2008.03.008

53. Bereket C, Ozan F, Sener I, Tech M, Altunkaynak B, Semirgin S: Propolis accelerates the consolidation phase in distraction osteogenesis. J Craniofac Surg (2014) 25: 1912-1916. doi: 10.1097/SCS.0000000000000946.

54. Ekeuku S, Chin K: Application of propolis in protecting skeletal and periodontal health-a systematic review. Molecules (2021) 26:3156. doi: 10.3390/molecules26113156.

55. Mansour S, Mossa A, Heikal T: Cytogenetic and hormonal alteration in rats exposed to recommended “safe doses” of spinosad and malathion insecticides. Int J of Agriculture and Biology (2008) 10: 9-14

56. Cemek M, Aymelek F, Büyükokuroğlu M, Karaca T, Büyükben A, Yilmaz F: Protective potential of Royal Jelly against carbon tetrachloride induced-toxicity and changes in the serum sialic acid levels. Food Chem Toxicol (2010) 48:2827-2832. doi: 10.1016/j.fct.2010.07.013.

الملخص العربى

تخفيف البروبوليس لسمية الميثوميل على أجنة الفئران وصغارها أثناء الحمل والرضاعة

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المقدمة: الميثوميل هو مبيد كرباماتي يستخدم على نطاق واسع، له العديد من التطبيقات في الزراعة بسبب فعاليته في مكافحة الآفات. ومع ذلك، أثار الاستخدام الواسع النطاق للميثوميل مخاوف بشأن آثاره الضارة المحتملة، خاصة أثناء الحمل. اكتسب البروبوليس، في السنوات الأخيرة ، وهو مادة راتنجية طبيعية يجمعها نحل العسل من مصادر نباتية مختلفة، اهتمامًا كبيرًا لخصائصه العلاجية المحتملة وتأثيراته التحسينية، خاصة أثناء الحمل.

الأهداف: كان الهدف من هذه الدراسة ، ذات النهج المزدوج ، تقييم التأثير المحتمل لإستخدام الميثوميل على الشكل والتطور الهيكلي الداخلي لأجنة الفئران وصغارها، وكذلك الانحرافات الكروموسوميه في امهات الفئران وصغارها. بالإضافة إلى ذلك، قمنا بدراسة التأثير التحسيني المحتمل للبروبوليس على السمية الناجمة عن الميثوميل.

مواد وطرق البحث: تم إعطاء الميثوميل عن طريق الفم بجرعة ٢ملغم/كغم، في حين تم إعطاء البروبوليس عن طريق الفم بجرعة ٣٠٠ملغم/كغم للأمهات الحوامل والمرضعات. تم تقسيم الفئران إلى ٤ مجموعات،)١) المجموعة الضابطة التي تلقت ١ مل من الماء المقطر عن طريق الفم،)٢) مجموعة البروبوليس، تلقت ١ مل من البروبوليس،)٣) مجموعة الميثوميل، تلقت ١ مل من الميثوميل و)٤) المجموعة المشتركة، تلقت ١ مل من الميثوميل يليه ١ مل من البروبوليس ع طريق الفم.

النتائج: أظهرت النتائج أن تعرض الأم للميثوميل أدى إلى حدوث العديد من التشوهات الشكلية والهيكلية الداخلية مثل الفتق السري، صغر حجم الأذن، رقة الجلد، صغر الفك، نزيف تحت الجلد و اعوجاج العمود الفقري، بالإضافة إلى التغيرات النسيجية في القطاعات العظمية لصغار الفئران وانخفاض التعظم القشري. بالإضافة إلى ذلك، تسبب الميثوميل في زيادة كبيرة في تكوين الكروموسومات الشاذة، والتي شملت التجزئة، والكروموسومات الحلقية، والكسر المركزي، والاندماج المركزي، والارتباط من طرف إلى طرف والفجوات الكروموسومية. ومن المثير للاهتمام أن إستخدام البروبوليس خفف وعكس بشكل واضح غالبية التأثيرات الضاره للميثوميل على أجنة الفئران وصغارها.

الخلاصه: بناء على هذه النتائج، يمكن استخدام البروبوليس كمكمل غذائي لتحسين وتقليل الآثار الضارة لتعرض الأم للميثوميل أثناء الحمل والرضاعة.