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Teratological study on the effect of methomyl on pregnant rats beside the protective effect of Curcuma longa on these dams

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Back grounds:
This study explained the hazardous effects of methomyl insecticide repeated exposure on pregnant female rats, raising concerns about this insecticide that possesses a potential hazard to dams and their foeti. It was concluded that *Curcuma longa* 100 mg/kg b.wt plays a very important role in ameliorating the adverse effects of methomyl. It is highly recommended to use *Curcuma longa* 100 mg/kg b.wt as a complementary agent for saving health from the hazards of any environmental pollutant, such as pesticides, during pregnancy.

ABSTRACT:

The goal of the current investigation was to determine whether methomyl, when given orally to female albino rats during organogenesis (from the sixth to the fifteenth day of gestation), was hazardous to development and whether *Curcuma longa* might have a protective effect against methomyl teratogenicity effects. The morphological analysis of the uterus and fetuses was evaluated. The number of implantation sites, resorption sites, pre-and post-implantation loss, and late resorption sites was observed. The fetuses were additionally checked for skeletal issues. The number of corpora lutea per dam treated with *Curcuma longa* increased significantly. Late resorption sites were significantly elevated in the single oral dose of methomyl equivalent to 1/20 LD_{50} when compared to the *Curcuma longa* groups. Compared to the *Curcuma longa* groups, a substantial increase in the percentage of post-implantation loss per dam and external abnormalities in fetuses derived from dams given methomyl were observed. The dose of methomyl (1/20 LD_{50}) groups was observed in the xiphosternum, wavy ribs, and partial or absent sternal ossification. From these findings, *Curcuma longa* plays a critical role in mitigating the effects of methomyl during the organogenesis phase.

Results:
After receiving a single dose of methomyl that was equal to 1/20 of LD50, female albino rats began to experience ocular hemorrhage, tremors, biliary retraction, paralysis, and convulsions. Respiratory failure is caused by respiratory muscle paralysis. Teratogenicity, which is defined as the induction or increased frequency of structural problems during pregnancy, is a specific instance of fetotoxicity. Any structural or functional abnormalities brought on by prenatal exposure to the inducing agent(s) are referred to as fetotoxicity. Research on the teratogenicity of any substance is typically conducted on rodents, particularly rats, because of their sensitivity to chemical effects, high fertility rates, low rates of spontaneous deformity, large number of litters, and short gestation periods.

Conclusion:
The effects of numerous drugs on reproductive components and functions are governed by an incredibly intricate process. Different substances may affect reproductive system components in a variety of ways. They may have an impact either directly by interfering with the reproductive components of the substance or indirectly by changing hormonal control. The cholinesterase enzymes are inhibited by the carbamate pesticides, including carbaryl, to kill insects. The main way that these pesticides harm mammals is through inhibition. The cholinesterase enzymes break down acetylcholine and other choline esters; thus, when they are inhibited, endogenous levels of acetylcholine and other choline esters go up. Inhibiting acetylcholinesterase causes an accumulation of endogenous acetylcholine, the main choline ester that has shown physiologic importance in humans, which is likely the cause of the majority of the biologic effects of anticholinesterase drugs like carbaryl. Analyze the harmful effects of methomyl on the biochemical parameters of female rats as well as its developmental toxicity. While 1/30 and 1/20 LD_{50} dosages of methomyl did not differ from control, the 1/10 LD_{50} dose reduced the relative weights of the ovary, uterus, and placenta.

Back ground

Methomyl is an O-(methyl carbamoyl) oxime carbamate; as such, its structure is similar to both aldicarb and thiocarbamide [1]. Methomyl is an oxime carbamate insecticide that controls a broad spectrum of arthropods such as spiders, ticks, moths, flies, beetles, aphids, leafhoppers, and spider mites often found on various field crops, ranging from fruits to tobacco [2]. Human exposures to methomyl fall into three toxicity categories defined by the USEPA that depend on the route of exposure: I, oral exposure (highly toxic); II, inhalation (moderately toxic); and III, dermal exposure (slightly toxic) [3]. Furthermore, methomyl is considered to be highly toxic to mammals, fish, and aquatic invertebrates [4]. To illustrate, the acute oral LD_{50} given for rats was 17–45 mg/kg [5]. At the same time, insecticides are considered potent pollutants in the environment that have been involved in birth defects and reproductive failure. The adverse health effects correlated with exposure to insecticides during pregnancy have become a considerable public health interest due to the widespread use of insecticides and the rising instability of the fetus and the pregnant mother to toxic exposures [6]. The metabolic pathway for methomyl in rats includes the displacement of the S-methyl moiety by glutathione and enzymatic transformation to produce a mercapturic acid derivative. Another pathway involves hydrolysis to give S-methyl-N-hydroxythioacetimidate, which is rapidly broken down to carbon dioxide [7]. Methomyl is rapidly metabolized to CO2 and acetonitrile. Methomyl-treated rats showed histopathological changes in the kidneys and spleens of male and female rats. Similarly, enzymatic alterations of acetylcholinesterase (AChE) and liver glucose-6 phosphate dehydrogenase were also observed [8]. The mixed-function oxidase system plays an important role in the metabolism of many compounds, including fatty acids, steroids, alkanes, polycyclic carcinogens, drugs, and environmental pollutants. Methomyl is a potent inhibitor of AChE, in both insects and mammals. The signs of toxicity are those expected from a cholinesterase (ChE) inhibitor, like profuse salivation, lacrimation, tremors, abnormal posture, pupil constriction, diarrhea, and prostration [7].

The use of many plants and herbs for fertility regulation, especially among women, has been prevalent in India for many centuries. Natural plant substances possessing mild inherent estrogenic or anti-estrogenic properties offer themselves as an effective non-conventional source of contraception with fewer deleterious side effects. Many plants and herbs have been reported to have potential antifertility properties [9]. *Curcuma longa* (English: turmeric), a member of the family Zingiberaceae, has been extensively used as a coloring agent, condiment, and in the treatment of inflammatory conditions and other diseases [10]. *Curcuma longa* (diferuloyl methane) bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione, which is a natural polyphenol alkaloid yellow-orange dye derived from the rhizome of C. longa, is known to exhibit a variety of pharmacological effects [11]. Traditional medicine has been recommended by the World Health Organization (WHO) as a cost-effective substitute for manufactured antifertility medicines. Parkes mouse strain was given aqueous rhizome extract of C. longa via the oral route (600 mg/kg body weight/day for 8 and 12 weeks), which causes reversible spermatogenesis, decreased seminiferous tubule diameter, and loosening of germinal epithelium, thus indicating its potential in male fertility [12]. Also examined was the influence of an aqueous C. longa rhizome extract on sperm count, spermatozoa motility, and seminal pH in Swiss Albino male mice, leading to infertility. The combined action of Curcuma longa and andrographolide significantly suppressed the number of implants and litter size in female Sprague-Dawley rats, changed the duration of phases involved in the estrus cycle, and lowered the number of ovarian follicles [13]. Petroleum ether, in addition to the aqueous extract of rhizome, shows an antifertility impact on rats via oral administration and results in complete inhibition of implantation. Curcuma longa also reduces human sperm motility, suggesting its usage as an intravaginal contraceptive and its antispermatogenic activity [14]. As stated in the literature cited, oxidative stress has been proposed as an alternative mechanism of pesticide toxicity, e.g., carbofuran toxicity in certain animal tissues, via impairment of the mitochondrial respiratory

system that leads to increased generation of free radicals through lipid peroxidation of the cell membrane, which plays a significant role not only in the pathogenesis of neuronal diseases but also in non-neuronal complications [15&16]. Since free radical generation is expected to induce organ toxicities, including hepatotoxicity and others, supplementation with antioxidants such as curcumin improves tissue capacity to cope with the high antioxidant demands [17&18]. Therefore, safe and effective natural products that may confer free radical scavenging activities are in global demand as an additional armamentarium against oxidative damage, especially for pregnant women [19]. This study aims to investigate the relationship between methomyl and Curcuma longa and its effect on pregnant rats and fetal teratogenicity with the anticonceptive activity of C. longa of this plant and its hormonal profile in immature bilaterally ovariectomized female rats to gain insight into its possible mode of action. This study aims to investigate the relationship between methomyl and Curcuma longa and its effect on pregnant rats and fetal teratogenicity with the anticonceptive activity of C. longa of this plant and its hormonal profile in immature bilaterally ovariectomized female rats to gain insight into its possible mode of action.

Materials

Methomyl [methyl, N-(methyl carbamoyloxy) ethanimidothioate] was provided from the Central Agricultural Pesticides Laboratory (CAPL), ARC, and Egypt. Methomyl possesses high acute toxicity in rats (LD_{50} of 30–34 mg/kg, equivalent to 1/20 LD_{50}) and is classified as a highly hazardous substance by the WHO [7]. *Curcuma longa* [(diferuloyl methane) bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-Dione] was obtained from the El-Ghomohria Company in Cairo, Egypt.

Experimental animals

The tested pesticide was prepared freshly in distilled water and administered as mg/kg b.w. by gavages. The method of [20] was used to estimate the oral LD_{50} of methomyl in female albino rats. Rattus norvegicus (Wister strain) females were received from Organization Biological Products and Vaccine, Helwan, Egypt, and kept in plastic cages until they were sexually mature and primiparous. Before the experiment, the experimental animals were left at least for two weeks to acclimatize in a lab setting (at a temperature of 25 ± 5 °C) and received a balanced diet (23% protein) and tap water. The female rats were kept in hygienic conditions. The females were considered mated on the day when sperm plus confide cells were found in the vaginal smear. This day is designated as the zero day of pregnancy [21]. The experiments were approved by the ARC-IACUC Committee of the Animal Health Research Institute, Agricultural Research Center. (ARC) [IACUC protocol Number (ARC-AH-22-15)].

Animal Treatment

The tested pregnant females were divided into eight equal groups, each consisting of five rats. The animals in the first group were kept as controls (untreated). The 2nd group was daily given an oral dose of methomyl equivalent to 1/20 high doses of LD_{50} from the 6th to the 15th day of gestation, and the 3rd group was daily given an oral dose of (500 mg/kg b.w.) *Curcuma longa* from the 6th to the 15th day of gestation. The 4th group was daily given an oral dose of (250 mg/kg b. w) *Curcuma longa* from the 6th to 15th day of gestation, the 5th group was daily given an oral dose of (100 mg/kg b. w) *Curcuma longa* from the 6th to 15th day of gestation, the 6th group was daily given an oral dose of *Curcuma longa* (500 mg/kg b. w) and methomyl equivalent to 1/20 of LD_{50}) from the 6th to 15th day of gestation, the 7th group was daily given an oral dose of *Curcuma longa* (250 mg/kg b. w) and methomyl equivalent to 1/20 of LD_{50} from the 6th the 15th day of gestation, and the 8th group was daily given an oral dose of *Curcuma longa* (100 mg/kg b. w) and methomyl equivalent to 1/20 of LD_{50} from the 6th the 15th day of gestation. All rats were sacrificed on the 20th day of gestation.

Developmental Toxicity

The development toxicity test was achieved to investigate how methomyl affects pregnant female rats' fetuses and embryos during organogenesis and fetogenesis. A hazardous exposure at this time is most likely to result in structural malformation. The period of embryogenesis is typically defined as the interval between the implantation and closure of the hard palate (from the 5th to the 16th day of gestation). Tissue differentiation, development, and physiological maturation

characterize the fetogenesis period. The development of these organs continues as the fetus develops the necessary functions before delivery. Nearly all organs are present and grossly visible. The likelihood of a hazardous exposure at this time is higher than the likelihood of developmental delays, functional changes, and decreased system capacity [22].

Morphological Examination of Uteri and Foeti of the Pregnant Female Rats

The methods of [21&23] were used for the morphological examination of the uterus and fetus of pregnant female rats. The uteri of the pregnant female rats that did not appear pregnant were meticulously examined by staining with ammonium sulfide for 10 to 20 min. The uterus of a pregnant female rat with attached ovaries was removed from the abdominal cavity and placed on dissection blotting paper. The number of corpora lutea in each ovary was counted. The uterine horns were opened, and the number and distribution of the implantation sites were recorded.

Examination of Foetal Skeleton

The eviscerated, skinned foeti were fixed in plastic bottles filled with 95% ethanol. Fixation of rat's foeti generally requires at least 7 days. After alcohol fixation period, the fetuses were placed into plastic compartments and the remaining alcohol was drained away. Each compartment was then filled with 1% KOH solution and the fetus were allowed to mascarate for approximately 24h. The foeti ready for staining when non-calcifed tissues are clear and the bones are cream-to buff-colored. After cleaning, the foeti were stained in Mallsch's solution with alizarin red S stain which is critical to proper visualization of bones for 24 h or until the bones are more vivid than the surrounding tissues [20]. The cleared fotei were transferred to Mallsch's solution with alizarin red S stain for 48 h to destain, followed by 20, 50, 20, and 100% of glycerin solution, respectively. The stained foeti were examined under dissecting microscope at a magnification 12.5X for any abnormalities in shape, size or absence of bones. The skull was examined for size, shape and degree of ossification by dissecting microscope. The fore-limbs and hind-limbs examined for development of the long bones [19].

Results

The External Symptoms of Methomyl Poisoning

After receiving a single dose of methomyl that was equal to 1/20 of LD_{50}, female albino rats began to experience ocular hemorrhage, tremors, biliary retraction, paralysis, and convulsions. Respiratory failure is caused by respiratory muscle paralysis.

Teratogenicity, which is defined as the induction or increased frequency of structural problems during pregnancy, is a specific instance of foetotoxicity. Any structural or functional abnormalities brought on by prenatal exposure to the inducing agent(s) are referred to as fetotoxicity [19].

Research on the teratogenicity of any substance is typically conducted on rodents, particularly rats, because of their sensitivity to chemical effects, high fertility rates, low rates of spontaneous deformity, large number of litters, and short gestation periods [17] (Figure 1).

Skeletal and visceral examination of rat fetuses

The data presented in Table 1 show that the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD50 during the period of organogenesis the lower dose (3.6%) and at the higher dose (19.2%) (Fig.5), Shortening of some ribs of such foeti was observed at the lower dose (11%) and at the higher dose (27%), (Fig.4). Also, wavy ribs were noticed at the higher dose (27%), (Fig.4).Split or absence of some sternebrae was observed at the lower dose (12.7%) and at the higher dose (23%) (Fig.2).In addition, absence of the xiphoid sternum was noticed at the lower dose (5.4%) and at the higher dose (9.6%).Absence of some bolex was observed at the lower dose (9.0%) and at the higher dose (15.3%). In addition, pelvic kidney was observed at the lower dose (14.5%) and at the higher dose (28.8%) (Fig.9).Also, Growth retardation was observed at the lower dose (32.7%) and at the higher dose (61.5%) (Fig.7). Reducing of heart, absence lobe of lung and

degeneration of liver was noticed at the lower dose (3.6 &7.2%) and at the higher dose (15.3&9.6%) (Fig.10). Microphilisima was noticed at the lower dose (3.6%) and at the higher dose (17.3%) (Fig.8).

Table 1: Embryonic weight, weight of organs, and embryonic length following treatment that only methomyl and associated doses of *curcuma longa*.

Groups	Fetal weight	Fetal length	Weight of placenta	Weight of liver	Weight of kidney	Weight of brain
Negative control	29.96± 4.8	30.90± 3.2	5.50± 4.8	10.06± 3.8	10.06± 3.8	1.48± 3.8
Meth1/20	16.48± 7.6[a]	20.92± 8.8[a]	2.64± 7.6[a]	6.82± 1.6[a]	1.22± 0.1[a]	1.56± 0.1
Cur 100mg/dl	33.24± 1.7[a]	31.02± 1.1[a]	7.18± 1.7[a]	10.82± 2.4	1.16± 0.1	1.24± 0.1
Meth 1/20 + Cur 100mg/dl	26.38± 4.6[b,c]	25.76± 3.4[b,c]	5.28± 4.6	9.26± 2.8	1.04± 0.1	1.06± 0.1
Cur 250mg/dl	23.66± 1.3[a]	26.06± 11.0[a]	3.82± 15.3[a]	9.02± 0.4	1.16± 0.1	1.58± 0.1
Meth1/20 + Cur 250mg/dl	21.10± 13.8[b,c]	22.64± 11.8[b,c]	3.18± 13.8[b]	7.98± 0.3[b,c]	0.96± 0.2[b,c]	1.50± 0.1
Curs 500mg/dl	17.46± 1.87[a]	25.02± 3.1[a]	3.02± 1.8[a]	8.28± 0.7[a]	1.02± 0.1	1.42± 0.1
Meth1/20 +Cur 500mg/dl	15.82± 1.81[a]	22.60± 2.0[b,c]	2.52± 1.8[b]	7.86± 0.6,c[b]	0.92± 0.1[b,c]	1.18± 0.1[c]

All data are expressed as means ±SD; a significant difference between methomyl treatment alone and control ($p<0.05$). a significant difference between methomly treatment with *curcuma longa* andcontrol (p<;0.05). a significant difference between methomyl treatment alone and methomyl treatment with curcumin ($p<0.05$). a significant difference between *curcuma longa* treatment alone and control ($p<0.05$).

The data presented in Table 1 show that the administration of the pregnant female rats with an oral dose of methomyl equivalent to 1/20 of the LD_{50} from the 6th to the 15th day of gestation resulted in statistically highly significant decreases ($p<0.05$), **(a)** in fetal weight, fetal length, and placenta Weight, liver weight, and kidney weight showed different significant decreases, while there was no significant difference in brain weight between methomyl treatment alone and control. Also, a significant decrease was found between methomyl treatment with *curcuma longa* and control ($p<0.05$). Fetal length, placenta weight, liver weight, and placenta weight, while not significant in brain weight, also found significant decreases between methomyl treatment alone and methomyl

Treatment with *curcuma longa* ($p<0.05$) **(c)** in fetal weight, fetal length, placenta weight, and liver weight, while no significant differences were seen in methomyl and *curcuma longa* 100 mg/dl. Also, the present data found a significant increase of different *curcuma longa* treatments alone and control ($p<0.05$) **(d)** an increase in significant *curcuma longa* 100 mg/dl in fetal weight, fetal length, placenta weight, liver weight, kidney weight, and brain weight, while significant decreases in *curcuma longa* 500 and 250 mg/dl in fetal weight, fetal length, placenta weight, and liver weigh (Fig. 10). Microphilisima was noticed at the lower dose (3.6%) and the higher dose (17.3%) (Fig. 8)

The data presented in Table 2 show that the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD_{50} with *Curcuma longa* (500) mg/kg b. wt. during the period of organogenesis (12%) and *Curcuma longa* (500) mg/kg b. wt. dose (0%), while the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD_{50} with *Curcuma longa* (250) mg/kg b. wt. during the period of organogenesis the (4.6%) and *Curcuma longa* (250) mg/ kg b. wt. dose (0%), while the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD_{50} with *Curcuma longa* (100) mg/ kg b. wt. during the period of organogenesis the (1.5%) and *Curcuma longa* (100) mg/ kg b. wt. dose (0%), Shortening of some ribs of such foeti was observed at the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD_{50} with *Curcuma longa* (500) mg/ kg b. wt. during the period of organogenesis the (17%) and *Curcuma longa* (500) mg/ kg b. wt. dose (7.9%), while the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD50 with *Curcuma longa* (250) mg/ kg b. wt. during the period of organogenesis the (4.6%) and *Curcuma longa* (250) mg/kg b. wt. dose (2.7%), while the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD50 with *Curcuma longa* (100) mg/kg b. wt.

during the period of organogenesis the (1.5%) and *Curcuma longa* (100) mg/kg b. wt. dose (0%).

Split or absence of some sternebrae was observed at the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD_{50} with *Curcuma longa* (500) mg/ kg b. wt. during the period of organogenesis the (12%) and Curcuma longa (500) mg/ kg b. wt. dose (0%), while the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD50 with Curcuma longa (250) mg/ kg b. wt. during the period of organogenesis the (4.6%) and Curcuma longa (250) mg/ kg b. wt. dose (0%), while the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD_{50} with Curcuma longa (100) mg/ kg b. wt. *Curcuma longa (100*) mg/kg b. wt. and Curcuma longa (100) mg/kg b. wt. dose (0%) during the period of organogenesis. In addition, the absence of the xiphoid sternum was noticed at the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD_{50} with Curcuma longa (500) mg/ kg b. wt. during the period of organogenesis the (6.9%) and Curcuma longa (500) mg/ kg b. wt. dose (3.1%), while the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD50 with Curcuma longa (250) mg/ kg b. wt. during the period of organogenesis the (3.1%) and Curcuma longa (250) mg/ kg b. wt. dose (1.4%), while the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD_{50} with Curcuma longa (100) mg/ kg b. wt. dose (3%), and Curcuma longa (100 mg/kg b. wt. dose) during the period of organogenesis. Absence of somebolex was observed at the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD50 with *Curcuma longa* (500) mg/ kg b. wt. during the period of organogenesis the (6.9%) and *Curcuma longa* (500) mg/ kg b. wt. dose (1.5%), while the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD_{50} with *Curcuma longa* (250) mg/ kg b. wt. during the period of organogenesis the (3.1%) and *Curcuma longa* (250) mg/ kg b. wt. dose (0%), while the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD_{50} with *Curcuma longa* (100) mg/ kg b. wt. during the period of organogenesis the (1.5%) and Curcuma longa (250) mg/ kg b. wt. dose (0%).

In addition, pelvic kidney was observed at the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD_{50} with *Curcuma longa* (500) mg/ kg b. wt. during the period of organogenesis the (8.6%) and *Curcuma longa* (500) mg/ kg b. wt. dose (1.5%), while the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD_{50} with Curcuma longa (250) mg/ kg b. wt. during the period of organogenesis the (4.6%) and *Curcuma longa* (250) mg/ kg b. wt. dose (1.4%), while the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD_{50} with Curcuma longa (100) mg/ kg b. wt. dose (1.5%) and *Curcuma longa* (100) mg/ kg b. wt. dose (0%) during the period of organogenesis. Also, Growth retardation was observed at the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD_{50} with Curcuma longa (500) mg/ kg b. wt. during the period of organogenesis the (34.5%) and Curcuma longa (500) mg/ kg b. wt. dose (15.8%),while the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD_{50} with Curcuma longa (250) mg/ kg b. wt. during the period of organogenesis the (15.6%) and *Curcuma longa* (250) mg/ kg b. wt. dose (10%), while the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD_{50} with *Curcuma longa* (100) mg/ kg b. wt. during the period of organogenesis the (7.5%) and *Curcuma longa* (100) mg/ kg b. wt. dose (0%)

parameter	G1 Methomyl 1/20		G2 Negative control		G3 Curcumin 500mg/dl		G4 Methomyl 1/20 + Curcumin 500mg/dl		G5 Curcumin 250mg/dl		G6 Methomyl 1/20 + Curcumin 250mg/dl		G7 Curcumin 100mg/dl		G8 Methomyl 1/20 + Curcumin 100mg/dl	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No. of fetus	52		67		63		58		70		64		65		66	
Ribs	14	27	0	0	5	7.9	11	17	2	2.7	3	4.6	0	0	1	1.5
Sternebrae	12	23	0	0	6	9.5	9	15.5	0	0	3	4.6	0	0	0	0
Xiphoid sternum	5	9.6	0	0	2	3.1	4	6.9	1	1.4	2	3.1	0	0	2	3
skull	10	19.2	0	0	0	0	7	12	0	0	3	4.6	0	0	1	1.5
somebolex	8	15.3	0	0	1	1.5	4	6.9	0	0	2	3.1	0	0	1	1.5
Pelvic kidney	15	28.8	0	0	1	1.5	5	8.6	1	1.4	3	4.6	0	0	0	0
Growth retardation	32	61.5	0	0	10	15.8	20	34.5	7	10	10	15.6	0	0	5	7.5
Reducing of heart	8	15.3	0	0	0	0	5	8.6	0	0	1	1.5	0	0	0	0
Absence lobe of lung	5	9.6	0	0	0	0	2	3.4	0	0	1	1.5	0	0	0	0
Microphilisima	9	17.3	0	0	1	1.5	4	6.9	0	0	1	1.5	0	0	0	0

Examination of external feature of the rats' foeti

A growth retardation of the rats' foeti on the 20th day of gestation whose mothers were treated with an oral dose of methomyl equivalent to 1/20 of LD_{50} during the period of organogenesis was noticed (Figure 2). Hematoma was noticed in the hind limb of the rats' foeti whose mothers were given an oral dose of methomyl (Figure 2), represents the control fetus with normal organs and phenotype, respect to hematoma on ventral side, arm and hind limb of rat's fetus of treated group. In another group whose mother was daily treated with an oral dose of methomyl equivalent to 1/20 of the LD_{50} with *Curcuma longa* (500 and 250 mg/ kg b.w) showed Hematoma on ventral side of nick of rat's fetus, Hematoma on beside ear of rat's fetus, and growth retardation of the rat's fetus (Figure 2). The group whose mother was daily treated with an oral dose of methomyl equivalent to 1/20 of the LD_{50} with *Curcuma longa* (500 mg/ kg b w) showed Hematoma on the arm of rat's fetus, Hematoma on ventral side of nick of rat's fetus, and Hematoma on ventral side of nick and hind limb of rat's fetus (Figure 2). Another treatment with an oral dose of *Curcuma longa* (250 mg/ kg b w) showed Hematoma on hind limb of rat's fetus (Figure 2). While, the group treated with an oral dose of *Curcuma longa* (100 mg/ kg b. w) showed Hematoma on the head and hind limb of rat's fetus (Figure 2).

Discussion

The effects of numerous drugs on reproductive components and functions are governed by an incredibly intricate process. Different substances may affect reproductive system components in a variety of ways. They may have an impact either directly by interfering with reproductive components of the substance or indirectly by changing hormonal control. The cholinesterase enzymes are inhibited by the carbamate pesticides, including carbaryl, to kill insects. The main way that these pesticides harm mammals is through this inhibition. The cholinesterase enzymes break down acetylcholine and other choline esters, thus when they are inhibited, endogenous levels of acetylcholine and other choline esters go up. Inhibiting acetylcholinesterase causes an accumulation of endogenous acetylcholine, the main choline ester that has shown physiologic importance in humans, which is likely the cause of the majority of the biologic effects of anticholinesterase drugs like carbaryl [21].

Xenobiotics provide evidence that it's crucial to assess both the active ingredient and the commercial formulation, which are what actually pose the greatest threat from agrochemicals [22, 23].

Analyze the harmful effects of methomyl on the biochemical parameters of female rats as well as developmental toxicity. While 1/30 and 1/20 LD_{50} dosages of methomyl did not differ from control, the 1/10 LD_{50} dose reduced the relative weights of the ovary, uterus, and placenta. Numerous hypothesised mechanisms exist for the anti-gonadal effects of carbamates [24].

The active component of the dietary spice turmeric is called curcumin (C. longa). In addition to being a hydrogen donor and free radical scavenger, Curcuma longa also has pro-oxidative and antioxidant properties. Additionally, it binds metals like iron and copper, making it a useful iron chelator. Curcuma longa has a remarkable lack of toxicity and a low bioavailability [25].

Methomyl has no effect on the presence of abnormalities since just one abnormality was seen in the foetuses of the treated women, in contrast to the study conducted by [26] in rats. Given that newborn infants may have serious deformities at birth or can be discovered later in life even without the presence of teratogens, the abnormality discovered is most likely not the product of methomyl. Resorption is the breakdown of a deceased foetus inside the uterus before it is partially or entirely reabsorbed [27]. According to [28], the ability of methomyl to increase the production of reactive oxygen species may have contributed to the foetuses in the treated groups' uteri dying. This suggests that methomyl's ability to cause oxidative stress may have had an impact on the foetuses' deaths [29].

According to [9], postictal administration of C. longa aqueous extract at a dose of 500 mg/kg body weight/day from days 1 to 5 of treatment prevented pregnancy in all treated female rats due to its mild estrogenic activity in the presence of a strong estrogen and its anti-implantation properties. Due to the multiple functions of the uterine biochemical environment.

According to [30] found that orally in doses of 250 mg/kg and 500 mg/kg, the ethanol extract of C. longa rhizomes had a significant hepatoprotective effect. The protective effect was dose-dependent. The capacity of turmeric and Curcuma longa to indirectly increase glutathione levels, assisting in liver detoxification, as well as direct antioxidant and free radical scavenging processes, may all contribute to their hepatoprotective properties [31]. C. longa and the volatile oils have strong anti-inflammatory properties [32].

Jain and Yadav (2006)[33] reported that oral administration of C. longa rhizome crude petroleum ether extract during early pregnancy (day 1–5 pc) at doses of 100, 200, and 500mg/kg b. w/day caused a significant adverse effect on pregnancy and the extract possesses potent (100%) pregnancy interceptor property at 500 mg/kg b. w/day dose level. The current results also show that oral administration of C. longa rhizome aqueous extract had a negative effect on fertility index and number of implantations in female rats at doses of 100, 250, and 500 mg/kg b. w/day from days 1 to 5 percent. This was due to an increase in the percentage of pre-implantation embryonic loss, which led to a decrease in the number of live foetuses. At 500 mg/kg b. w, however, a full (100%) blocking of pregnancy was seen since none of the mated females displayed the presence of an implantation site.

[28] Reported that administration of this plant's petroleum ether, alcoholic (95%) and aqueous extracts at dosages of 100 and 200 mg/kg on days 1–7 of pregnancy shown notable antifertility action. The results of the current investigation thus suggested that the aqueous extract of C. longa rhizome has anti-implantation activity, and that this anti-conceptive action may be caused, at least in part, by the extract's low estrogenic properties. However, haematological tests done on rats given extract treatment did not reveal any negative effects.

Fetal osteogenesis is a two-step process that starts with either an endochondral or intramembranous method of bone production, then lengthens and thickens compact bone. Osteogenesis needs calcium and other substances, which the placenta provides from the mother's blood circulation. Since every foetal

long bone has significantly shrunk. Therefore, it is comforting to know that the reduction in maternal bone resorption was what limited the calcium supply to foetuses, which in turn constrained their skeletal development [34].By lowering the supply of calcium and magnesium ions to the developing foetus, pesticides may have an impact on calcium metabolism and/or bone morphometry, delaying the growth of bones and, as a result, affecting the weight of the foetus [35]. Reduced foetal weights in the current study suggest that the delay in ossification of the skeletal system is linked to a delay in foetal growth. Similar findings have linked foetuses' growth retardation to the skeletal system's deficient ossification [36]. Placental weight loss occurring along with foetal weight loss [37]. Reduced blood supply from a smaller placenta is likely to cause severe foetal hypoxia, which could slow intrauterine growth. Endometrial biopsy (EMB) exposed mice showed a considerable loss in placental weight, which may have an impact on the placenta's nutritional function and cause resorption or at the very least growth retardation.

Gross structural malformations are most likely to result from toxic exposure during the first trimester of pregnancy (embryogenesis) or during the period of organogenesis, whereas toxic exposure during the later stages of pregnancy (fetogenesis) is more likely to be linked to stunted growth and functional alterations [19].

In the current study, dose of methomyl administered to pregnant rats throughout the carbamate phase resulted in significantly higher numbers of corpora lutea in each ovary and blastocyst implantation sites per dam. Additionally, at low doses of methomyl, there were demonstrable increases in the quantity of late resorptions and the percentage of post-implantation loss per dam.

Among the emerging contaminants, pharmaceuticals (hormones, antibiotics, and others), cosmetics, synthetic dyes, and pesticides are of the major concern in many areas around the world) [38], an amalgam of organophosphorous insecticides, and quinalphos [39, 40]. A number of studies, showed that pregnant rats treated with levalnil, chordimeform, deltamethrin, perchloroethylen, chlordane, heptachlor, cabaryl, monocrotophos, or carbosulfan experienced significant. In the current investigation, methomyl administration caused a significant decrease in the number of living foeti and an increase in the number of dead foeti in pregnant rats [41-44].

In the present study, the treatment of the pregnant rats with methomyl during the period of organogenesis induced significant decreases in the means of foetal weight and length. The results of the present study showed that the foeti obtained from methomyl-treated dams exhibited growth retardation. The results of the present study indicated that the maternal exposure to methomyl during the period of organogenesis might produce selective degree of risk to the developing foeti, which was implicated in a significant increase in the number of malformed foeti. Examination of rats, foeti whose mothers treated with methomyl during the period of organogenesis showed morphological malformations such as haematoma in different parts of the body and exencephaly, besides, skeletal malformations such as wavy ribs, shortening of some ribs, retarded development of some sternebrae or complete absence of sternebrae and delayed ossification of the skull.

In the present study, the direct exposure to the carbamate substance methomyl and its metabolites, which can cross the placental barrier, is responsible for several congenital abnormalities. In the current investigation, methomyl administration caused significant induced wavy ribs abnormally and induced delayed ossification of the skull of the treated rats.

The placenta is crucial to the development of the foetus because it supplies nutrients, regulates hormones, and transports metabolic waste. According to [45], carbamate pesticides can cross the placental barrier, increasing the likelihood that they will directly affect the tissues of the developing foetus.

The placenta may be directly involved in many cases of early spontaneous abortion, foetal mortality, and growth retardation because the buildup of toxic chemicals may change placental functioning and harm foetal development [46].

Conclusion

This study explained the hazardous effects of methomyl insecticide repeated exposure on the pregnant female rats, raising concerns about this insecticide that possesses a potential hazard to dams and there foeti .It was concluded that Curcuma longa 100 mg/kg b w play very important role in amelioration of the adverse effect of methomyl. It is highly recommended using of Curcuma longa 100 mg/kg b w was a complementary agent for saving health from hazards of any environmental pollutant such as pesticides during pregnancy.

Abbreviation

ACHE Acetyl cholinesterase

CHE Cholinesterase

ARC Agricultural Research Center

CAPL Central Agricultural Pesticides Laboratory

-Ethical statement

The study involves work with live animal's subjects as approved with a committee.

-Conflict of Interest

The authors declare no conflict of interest.

Funding

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Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Supplementary Material

No supplementary data are provided

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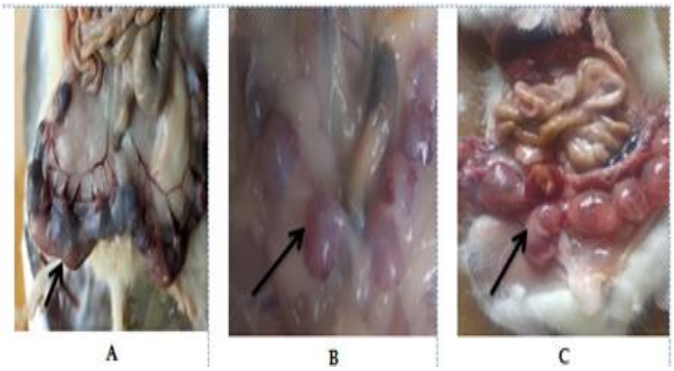


Figure 1: Teratogenic effect of a single dose of methomyl equal to 1/20 of the LD50 on rat fetuses with abnormal fetuses a: normal fetus in horn; b: early resorption; c: abnormal fetus (late resorption and growth retardation).

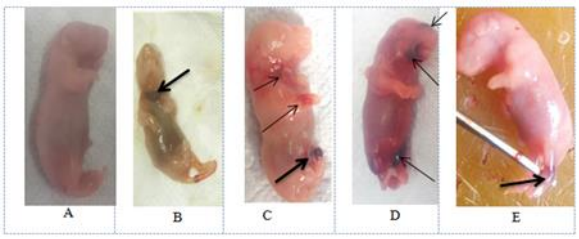


Figure 2: Examination of external features of the rats' fetus whose mother was daily treated with an oral dose of methomyl equivalent to 1/20 of the LD_{50} from the 6^{th} to the 15^{th} day of gestation and sacrificed on the 20^{th} day of gestation, oral dose of methomyl equivalent to 1/20 of the LD_{50} with *Curcuma longa* (500 and 250 mg/kg bw), oral dose of methomyl equivalent to 1/20 of the LD_{50} with *Curcuma longa* (500 mg/kg bw), oral dose of *Curcuma longa* (250 mg/kg bw), and oral dose of *Curcuma longa* (100 mg/kg bw) (A) Control fetus; (B, C, D, and E, respectively) hematoma on the ventral side, arm, hind limb, and nose of the rat's fetus.

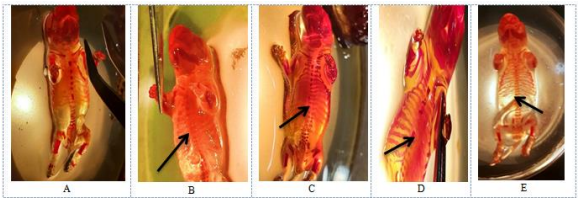


Figure3: Examination of foetal skeleton whose mother was daily treated with an oral dose of methomyl equivalent to 1/20 of the $LD_{50}$from the 6^{th} to the 15^{th} day of gestation and sacrificed on the 20^{th} day of gestation, oral dose of methomyl equivalent to 1/20 of the LD_{50} with *Curcuma longa* (500 & 250 mg/ kg b w), oral dose of methomyl equivalent to 1/20 of the LD_{50} with *Curcuma longa* (500 mg/ kg b w), oral dose of *Curcuma longa* (250 mg/ kg b w) and oral dose of *Curcuma longa* (100 mg/ kg b w) . (A) Control fetus (untrated), (B, C, D and E respectively) and absence of all streabrea, Split of 4^{rd} and 5^{rd} sternbrae of the rat's, absence of the 5^{th} and xiphoid of sternbrae of the rat's, absence of the 4^{rd} and 5^{th} and xiphoid of sternbrae of the rat's, split of 3^{rd} and 4^{th} sternebrae and absence of the 2^{nd} and the 5^{th} stearnbrae of rats' foeti.

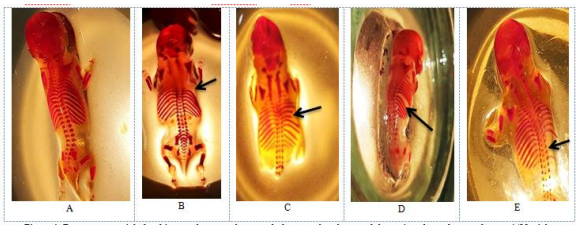


Figure4 : Examination of skeletal foetie whose mother was daily treated with an oral dose of methomyl equivalent to 1/20 of the LD_{50} from the 6^{th} to the 15^{th} day of gestation and sacrificed on the 20^{th} day of gestation, oral dose of methomyl equivalent to 1/20 of the LD_{50} with *Curcuma longa* (500 and 250 mg/kg b w), oral dose of methomyl equivalent to 1/20 of the LD_{50} with *Curcuma longa* (500 mg/kg b w), oral dose of *Curcuma longa* (250 mg/kg b w), and oral dose of *Curcuma longa* (100 mg/kg b w) from the 6^{th} to the 15^{th} day of gestation and sacrificed on the 20^{th} day of gestation (A) Control fetus (B,C, D, and E, respectively) absences of 3, 4,

and 5th of ribs shortening of the 6th and 7th of the ribs of the rat's fetus was visible in the shortening of the 11th, 12th, and 13th ribs and wavy ribs and the absence of the 10th, 11th, and 12th of the ribs.

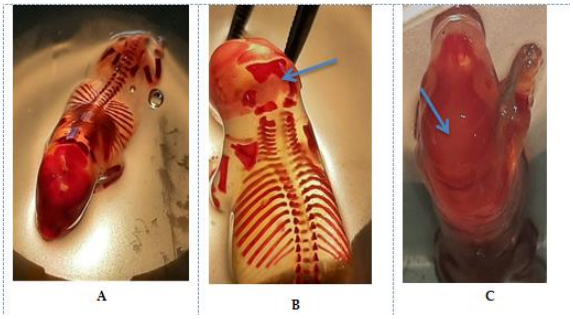


Figure 5: Examination of skeletal fetus whose mother was daily treated with an oral dose of methomyl equivalent to 1/20 of the LD_{50} from the 6th to the 15th day of gestation and sacrificed on the 20th day of gestation, oral dose of methomyl equivalent to 1/20 of the LD_{50} with *Curcuma longa* (500 and 250 mg/kg b w), oral dose of methomyl equivalent to 1/20 of the LD_{50} with *Curcuma longa* (500 mg/kg b w), oral dose of *Curcuma longa* (250 mg/kg b w), and oral dose of *Curcuma longa* (100 mg/kg b w) from the 6th to the 15th day of gestation and sacrificed on the 20th day of gestation. (A) Control fetus; (B&C) decreased ossification.

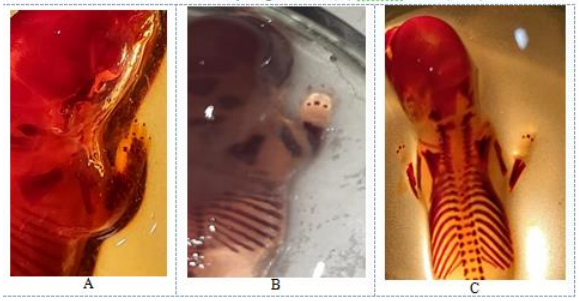


Figure 6 : Examination of skeletal foetie whose mother was daily treated with an oral dose of methomyl equivalent to 1/20 of the LD_{50} from the 6th to the 15th day of gestation and sacrificed on the 20th day of gestation, oral dose of methomyl equivalent to 1/20 of the LD_{50} with *Curcuma longa* (500 and 250 mg/kg b w), oral dose of methomyl equivalent to 1/20 of the LD_{50} with *Curcuma longa* (500 mg/kg b w), oral dose of *Curcuma longa* (250 mg/kg b w), and oral dose of *Curcuma longa* (100 mg/kg b w) from the 6th to the 15th day of gestation and sacrificed on the 20th day of gestation. (A) Control fetus; (B&C) absences of somebolex.

Figure 7 : Examination of fetal visceral whose mother was daily treated with an oral dose of methomyl equivalent to 1/20 of the LD50 from the 6th to the 15th day of gestation and sacrificed on the 20th day of gestation, oral dose of methomyl equivalent to 1/20 of the LD_{50} with *Curcuma longa* (500 and 250 mg/kg b w), oral dose of methomyl equivalent to 1/20 of the LD_{50} with *Curcuma longa* (500 mg/kg b w), oral dose of *Curcuma longa* (250 mg/kg b w), and oral dose of *Curcuma longa* (100 mg/kg b w) from the 6th to the 15th day of gestation and sacrificed on the 20th day of gestation. (A) Control fetus, and (B) growth retardation of the rat's fetus.

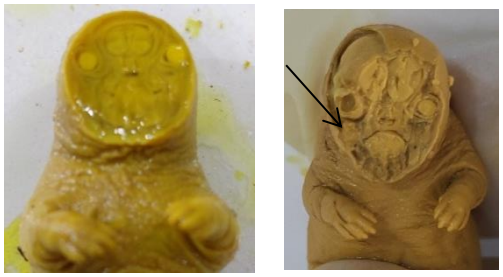
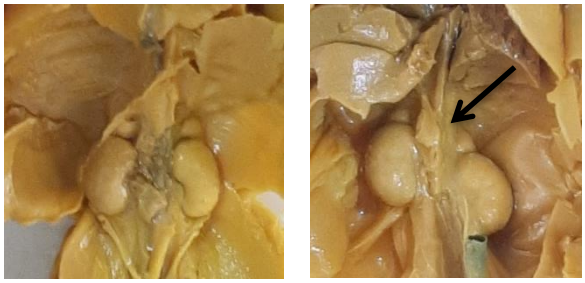


Figure8: Examination of fetal visceral whose mother was daily treated with an oral dose of methomyl equivalent to 1/20 of the $LD_{50}$from the 6th to the 15th day of gestation and sacrificed on the 20th day of gestation, oral dose of methomyl equivalent to 1/20 of the LD_{50} with *Curcuma longa* (500 & 250 mg/ kg b w), oral dose of methomyl equivalent to 1/20 of the LD_{50} with *Curcuma longa* (500 mg/ kg b w), oral dose of *Curcuma longa* (250 mg/ kg b w) and oral dose of *Curcuma longa* (100 mg/ kg b w)from the 6th to the 15th day of gestation and sacrificed on the 20th day of gestation. (A) Control fetus, and (B) micro anaphthalmia of rat's fetus.



A B

Figure 9 : Examination of fetal visceral whose mother was daily treated with an oral dose of methomyl equivalent to 1/20 of the LD50 from the 6th to the 15th day of gestation and sacrificed on the 20th day of gestation, oral dose of methomyl equivalent to 1/20 of the LD_{50} with *Curcuma longa* (500 and 250 mg/kg b w), oral dose of methomyl equivalent to 1/20 of the LD_{50} with *Curcuma longa* (500 mg/kg b w), oral dose of *Curcuma longa* (250 mg/kg b w), and oral dose of *Curcuma longa* (100 mg/kg b w) from the 6th to the 15th day of gestation and sacrificed on the 20th day of gestation. (A) Control and (B) pelvic kidney of the rat's fetus.

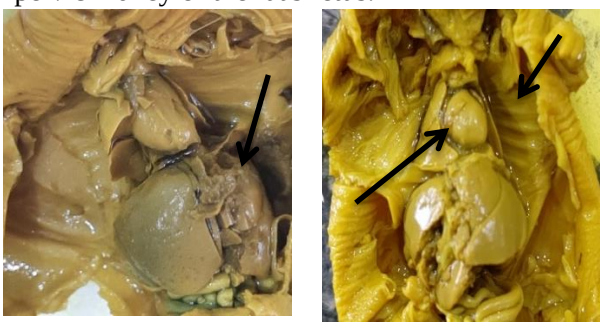


Figure 10 : Examination of fetal visceral whose mother was daily treated with an oral dose of methomyl equivalent to 1/20 of the LD_{50}, methomyl to 1/20 of the LD_{50} with *Curcuma longa* (250 and 500 mg/kg b w) and (250 and 500 mg/kg b w) of *Curcuma longa* from the 6th to the 15th day of gestation and sacrificed on the 20th day of gestation. absense of left lobe lung, then reduced heart, reducing heart, and degeneration of liver in the rat's fetus.