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## Research Article

# Fetal Gross Anomalies Induced by Prenatal Exposure of Khat *(Catha edulis*) in New Zealand Rabbits

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**ABSTRACT**

The current work was done on 27 adult apparently healthy New Zealand rabbits (24 female and 3 males). The extract of khat was orally administered to pregnant rabbits from 8th to 18th day of gestation at doses of 0, 3, 6 and 12 ml/kg. body weight. On 28th day of gestation, all dams were slaughtered and the fetal gross anomalies were assessed. The prenatal exposure to khat caused a decrease in length and weight of the fetuses. Cleft palate, meningeocele, twisted limb, umbilical hernia, resorbed feti and degenerated brain also were observed in the fetuses from khat treated dams. The present study indicated that khat possesses teratogenic properties on developing embryos. Also, we concluded that the developmental anomalies of khat are dose-related.

**Key words:** Khat, Rabbit, Foetus, Anomalies

## INTRODUCTION

Khat, *Catha edulis* Forsk (family celestraceae) is a flowering evergreen shrub or small tree that either grows wild or cultivated in certain regions of East Africa and Southern Arabia (Osborne 1983 and Carlini 2003). There are about 44 different types of Khat in Yemen Arab Republic (Al-Motarreb, Baker *et al.*, 2002).

Chewing Khat during pregnancy may contribute to infant death. Khat affects the potency of male sexuality by affecting spermatogenesis and plasma testosterone concentration(Mwenda *et al.*, 2003). Fresh khat leaves contain an amphetamine-like alkaloidcalled cathinone (Al-Ahdal *et al.*, 1988).

Khat also caused lethal mutations in mice (Li and Lin 1998) and embryotoxic and teratogenic effects in rats(Islam *et al.*, 1994). Khat has a genotoxic effect as it induce formation of micronuclei in human buccal and bladder mucosa(Kassie *et al.*, 2001).

## MATERIALS AND METHODS

### Experimental animals

The present work was carried out on (27) adult, apparently healthy New Zealand rabbits sexually mature of both sexes (3 males & 24 females). The animals ranged from (2.5±0.5 kg), body weight and were obtained from - Abu Sawyer Farm, Ismailia, Egypt.

### Place

The animals were housed in specially prepared steel cages, cleaned carefully with water and soap then, disinfected by phenol and left for two days and rinsed with water. The room was rinsed with iodine. Light was for 12H during night. The windows were kept after (shering ham system)

### Feed

Commercial pelleted food (New Tanpool Feed, Tanpool factory for rabbits feed, Al-mohnds, Egypt) and khat were tested free of any mycotoxins at the Central Laboratory of Residue Analysis of Pesticides and Heavy metals in food, Agricultural Research Center, Ministry of Agricultural, EGYPT. Food and water were given ad-libitum for both males and females.

### Acclimatization

Acclimatization periods were for two weeks. During this period, the animals were administered feed and water ad-lib with addition of vitamin E and selenium in water at a dose of (1 gm / litter) as immunostimulant and anti-stress.

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**Mating**

The females were placed in the cages of males at evening and mating was observed. Male grunting sound as well as turning to its side after mating indicated successful mating. Females were moved to its cage next day morning and considered that as day zero of pregnancy (Wangikar *et al*., 2005).

**Plant material**

Fresh khat (*Catha edulis)* leaves harvested in September 2014 from private farm Dhamar, Wedy alher. Governorate (Republic of Yemen). The plants were cut and dried and grind. Khat was tested for free Residue of Pesticides and Heavy metals in Agricultural Research Center, Ministry of Agricultural, Egypt.

**Preparation of khat extract:** One hundred grams of dried khat leaves were soaked in 500 ml of distilled water for 24hours and heated for 30 min at 60°C in a water bath. The contents were filtered using a piece of cloth to give about 300 ml extract which after that was chilled in refrigerator until use (Al-Mehdar *et al.*, 2012; Al-Qirim *et al*., 2002).

Dose selection was adopted based on that the average daily consumption of khat leaves is 2 gm per kg body weight (Alrajhi1 and Yousef (2013). Each 1 gm khat gave off 3ml khat extract so we use the three doses 3, 6 and 12 ml extract/ kg body weight.

**Method of administration**

Oral route of administration of (Khat) to pregnant rabbits was selected to ensure the administration of exact amount of Khat extract to the animals and was considered to be the most accurate way to give a fixed dose of developmental disorders induced by food borne Khat, Alrajhi and Yousef (2013).

**Experimental grouping**

The female animals were divided into four groups.

- The control group formed of (6) rabbits were given distilled water only and slaughtered at 28th day of gestation.
- The low dose group formed of (6) were given 3 ml extract per 1kg body weight per day from 8th - 18th day of gestation. Then they were slaughtered at day28th of gestation.
- The medium dose group formed of (6) animals were given 6 ml extract per 1kgm body weight per day form (8th -18th) day of gestation and slaughtered at 28th day of gestation.
- The high dose group formed of (6) animals were give 12 ml extract per 1kgm body weight per day form (8th -18th) day of gestation and slaughtered at 28th day of gestation.

**Observation**

On 28$^{th}$day of gestation, the rabbit was anaesthetized by intramuscular injection of a mixture of 30 mg xylazine (Rompun®, Butler, Columbus, OH, USA) and 1 mg acepromazine maléate (Tech America, Elwood, KS, USA) (Carney and Foote, 1990) then lapara to me was performed then the fetal swellings were recorded and the uterus was excised for removal of the feti to be examined for gross anomalies and the uteri were examined for any abnormality in the placenta.

## RESULTS

The present study showed that the administration of khat to pregnant dams during organogenesis produced an adverse effect on fetal growth and induced teratogenesis in addition to fetal resorption Table 1. The feti of khat treated dams showed a significant decrease in the fetal weight and CVRL (Tables 2, 3, 4 and 5) and Fig. 1 and 2.

One fetus 4.1% in the low dose group showed opened roof of the cranium and most of the roof is cartilaginous. and degeneration of most of the brain in one fetus 4% of same group Fig. 13. Meningeoceale was observed in one fetus 4% of low dose group Fig. 11. One fetus 5% in medium dose group showed a Congestion of cerebral veins Fig. 6. Subcutaneous congestion in trunk and pelvic limbs was observed in one fetus 5% of medium dose group Fig. 7. Diffused subcutaneous hemorrhage occurred in five feti 33% of the high dose group Fig. 7.

Twisting of the fore and hind limbs also were observed in one fetus of the medium dose group 5% and one fetus in high dose group 7% Fig. 8. Resorbed feti appeared in all treated groups; one fetus in low dose group 4%, two feti in medium dose group 10% and three feti in high dose group 20% Fig. 9. One fetus in low dose group 4% and one fetus in middle dose groups 5% showed incomplete fusion of the lateral and medial nasal

**Table 1:** Showing number and percentage of gross anomalies in fetus of khat treated rabbits.

| Group | No | Opened roof of the cranium | | Congestion in head, trunk and limbs | | Cleft palate and lip. | | Twisted limbs | | Resorbed feti | | Meningeoceale and hernia | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No | % | No | % | No | % | No | % | No | % | No | % |
| Control | 29 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Low dose | 24 | 1 | 4 | 1 | 4 | 1 | 4 | 0 | 0 | 1 | 4 | 1 | 4 |
| Middle dose | 20 | 0 | 0 | 2 | 10 | 0 | 0 | 1 | 5 | 2 | 10 | 0 | 0 |
| High dose | 15 | 0 | 0 | 5 | 33 | 0 | 0 | 1 | 7 | 3 | 20 | 0 | 0 |

**Table 2:** Showing the mean weight per grams feti of control and khat treated dams. low dose, medium dose, high dose.

| | N | Mean of feti weight | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Control | 29 | 45.0455 | 6.24936 | 1.16048 | 42.6684 | 47.4226 | 36.21 | 57.20 |
| low dose | 24 | 40.1500 | 3.47275 | .70887 | 38.6836 | 41.6164 | 30.00 | 48.60 |
| medium dose | 20 | 34.8450 | 2.25330 | .50385 | 33.7904 | 35.8996 | 30.50 | 39.70 |
| high dose | 15 | 29.6400 | 1.60036 | .41321 | 28.7538 | 30.5262 | 26.80 | 33.00 |

**Table 3:** Showing comparison of the weight per grams feti of control and khat treated dams. low dose, medium dose, high dose.

| Group (I) | | Group (J) | Mean difference (I-J) | SE | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| LSD | Control | low dose | 4.89552* | 1.16736 | .000 | 2.5741 | 7.2170 |
| | | medium dose | 10.20052* | 1.22958 | .000 | 7.7554 | 12.6457 |
| | | high dose | 15.40552* | 1.34541 | .000 | 12.7300 | 18.0810 |
| | low dose | control | -4.89552-* | 1.16736 | .000 | -7.2170- | -2.5741- |
| | | medium dose | 5.30500* | 1.28079 | .000 | 2.7580 | 7.8520 |
| | | high dose | 10.51000* | 1.39237 | .000 | 7.7411 | 13.2789 |
| | medium dose | control | -10.20052-* | 1.22958 | .000 | -12.6457- | -7.7554- |
| | | low dose | -5.30500-* | 1.28079 | .000 | -7.8520- | -2.7580- |
| | | high dose | 5.20500* | 1.44493 | .001 | 2.3316 | 8.0784 |
| | high dose | control | -15.40552-* | 1.34541 | .000 | -18.0810- | -12.7300- |
| | | low dose | -10.51000-* | 1.39237 | .000 | -13.2789- | -7.7411- |
| | | medium dose | -5.20500-* | 1.44493 | .001 | -8.0784- | -2.3316- |

*. The mean difference is significant at the 0.05 level.

**Table 4:** Showing the length of CVRL /cm of feti control and khat treated dams. low dose, medium dose, high dose.

| | N | CVRL Mean | Std. Deviation | SE | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| Control | 29 | 11.7034 | .85960 | .15962 | 11.3765 | 12.0304 | 10.00 | 13.50 |
| low dose | 24 | 10.5042 | .49737 | .10152 | 10.2941 | 10.7142 | 9.90 | 11.60 |
| medium dose | 20 | 9.8025 | .30670 | .06858 | 9.6590 | 9.9460 | 9.30 | 10.60 |
| high dose | 15 | 9.3267 | .34737 | .08969 | 9.1343 | 9.5190 | 9.00 | 9.90 |

**Table 5:** Showing comparison of CVRL/cm of feti control and khat treated dams. low dose, medium dose, high dose

| Group (I) | | Group (J) | Mean difference (I-J) | SE | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| LSD | control | low dose | 1.19928* | .16452 | .000 | .8721 | 1.5264 |
| | | medium dose | 1.90095* | .17328 | .000 | 1.5564 | 2.2455 |
| | | high dose | 2.37678* | .18961 | .000 | 1.9997 | 2.7538 |
| | low dose | Control | -1.19928-* | .16452 | .000 | -1.5264- | -.8721- |
| | | medium dose | .70167* | .18050 | .000 | .3427 | 1.0606 |
| | | high dose | 1.17750* | .19623 | .000 | .7873 | 1.5677 |
| | medium dose | Control | -1.90095-* | .17328 | .000 | -2.2455- | -1.5564- |
| | | low dose | -.70167-* | .18050 | .000 | -1.0606- | -.3427- |
| | | high dose | .47583* | .20363 | .022 | .0709 | .8808 |
| | high dose | control | -2.37678-* | .18961 | .000 | -2.7538- | -1.9997- |
| | | low dose | -1.17750-* | .19623 | .000 | -1.5677- | -.7873- |
| | | medium dose | -.47583-* | .20363 | .022 | -.8808- | -.0709- |

*. The mean difference is significant at the 0.05 level.

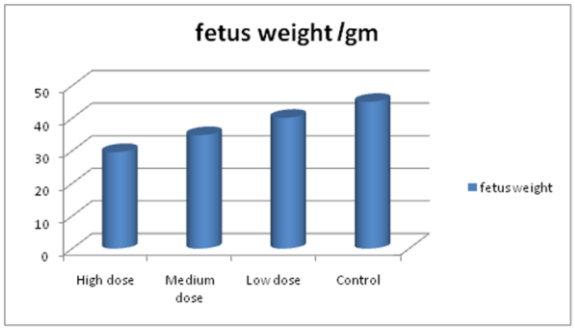


**Fig. 1:** A histogram showing the weight per grams of feti of control and khat treated dams.

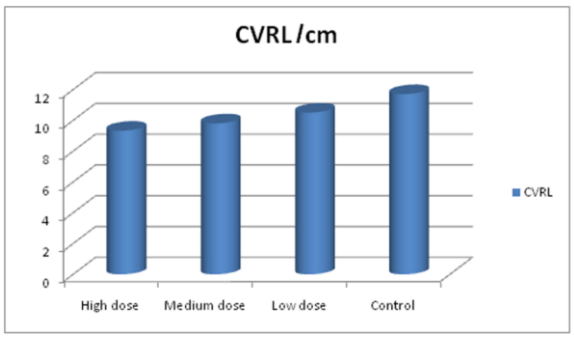


**Fig. 2:** A histogram showing the length of CVRL/cm of feti of control and khat treated dams.

prominences in addition to cleft lip Fig. 10. One fetus 4% in the low dose group showed cleft palate. Umbilical hernia was observed in one fetus of low dose group 4% Fig. 12.

## DISCUSSION

The present study recorded reduction of length and weight of feti of khat treated dams of all treated groups. These findings resembled the observations of Stewart and Meeker (1997) and Islam *et al*. (1994) in rat andYoung and Widdowson (1975)in guinea pig and Abd-El-Aziz (1998) in human. This reduction of weight may be due to depression of the appetite of the dam or the khat may decrease the rate of development (Mwenda *et al*., 1988) in human. In accordance with Isalm *et al*. (1994) in rat, the current study revealed that the administration of khat produced a significant increase in the number of resorptions and fetal wastage per litter in all tested doses.

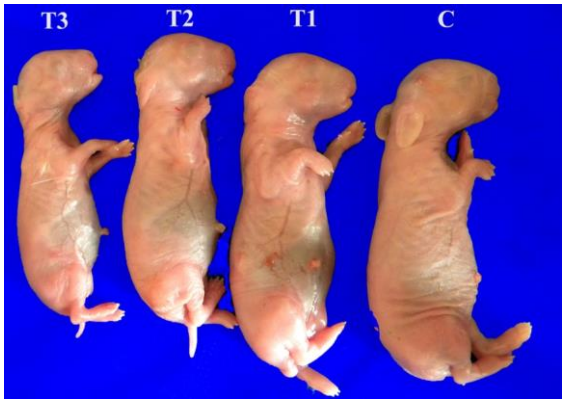


**Fig. 3:** A photograph of the feti at 28th day of gestation of control (c) and khat treated dams : low dose (T1), medium dose (T2) and high dose (T3).

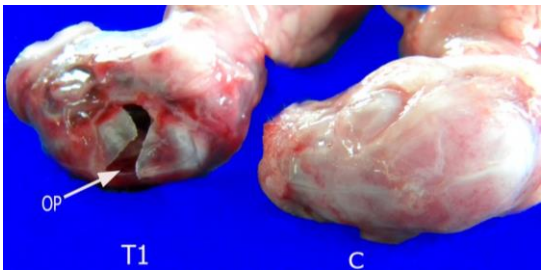


**Fig. 4:** A photograph of control rabbit fetus (C) and fetus of khat treated dams: low dose (T1), showing opened roof of the cranium and most of the roof is cartilaginous in addition to agenesis of most of the brain(OP).

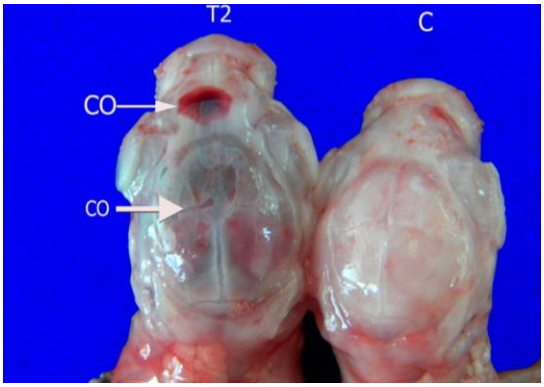


**Fig. 5:** A photograph of control fetus (C) and fetus of khat treated dams: medium dose (T2), showing Congestion of cerebral veins (CO).

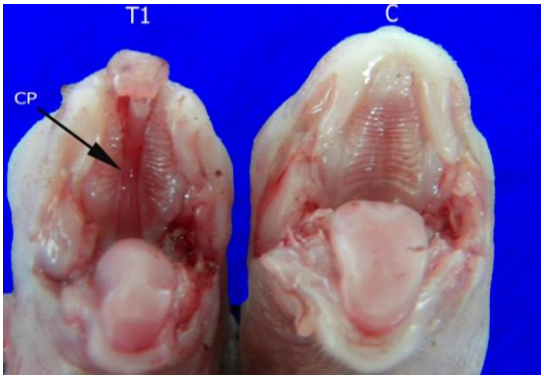


**Fig. 6:** A photograph of control fetus (C)and fetus of khat treated dams: low dose (T1), showing cleft palate (CP).

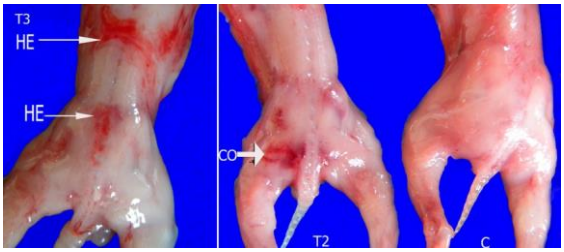


**Fig. 7:** A photograph of control fetus (C)and fetus of khat treated dams: medium dose (T2), high dose (T3), showing subcutaneous congestion(CO) and diffused subcutaneous hemorrhage (HE).

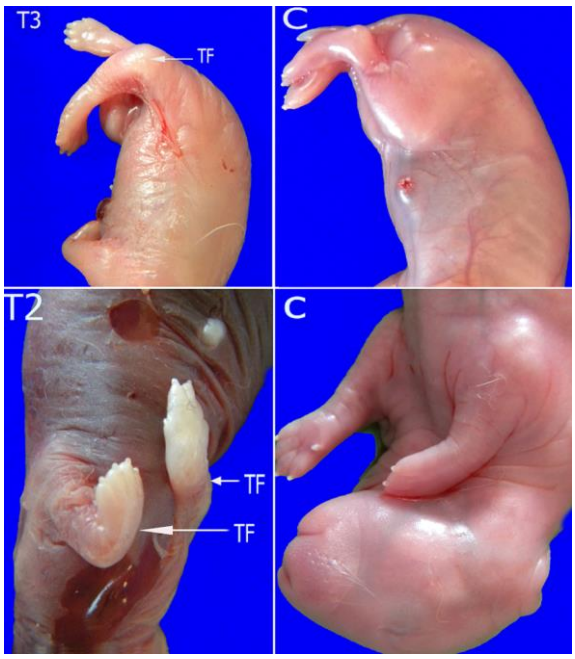


**Fig. 8:** A photograph of control fetus (C)and treated dams feti : medium dose (T2), high dose (T3), showing twisted fore and hind limbs) (TF).

The current study observed decrease in fetal weight and length in addition to increase in fetal resorption with the increase in dose of khat in rabbit while Yamamoto (1992) recorded increase in fetal malformation and mortality with increase in dose in methamphetamine treated mice. In our results, there were opened roof of the cranium and most of the roof is cartilaginous in addition to degeneration of most of the brain in one rabbit fetus in low dose group but this result did not record by Isalm *et al.*, 1994 in rat.

Cleft lip, twisted fore and hind limbs, cleft palate, were observed in some feti of treated rabbit dams. This result agreed with that of (Isalm *et al.*, 1994) in rat. Al-Meshal (1987) stated that, due to the complex nature of the khat extract, it is difficult to establish the exact nature of component(s) responsible for its teratogenic effects. But the current study tested the khat for pesticides so any teratogenic effect caused by the extract indicate the effect of khat only. The current study also showed that khat can impair fetal growth. These findings were in accordance with Abd-El-Aziz and Ahmed (1998) in human. Also hernia, meningeoceal and degeneration of the brain in low dose feti were found but this result did not recorded by Isalm *et al* 1994 in rat, Young and Widdowson (1975)in guinea pig and Abd-El-Aziz (1998) in human.

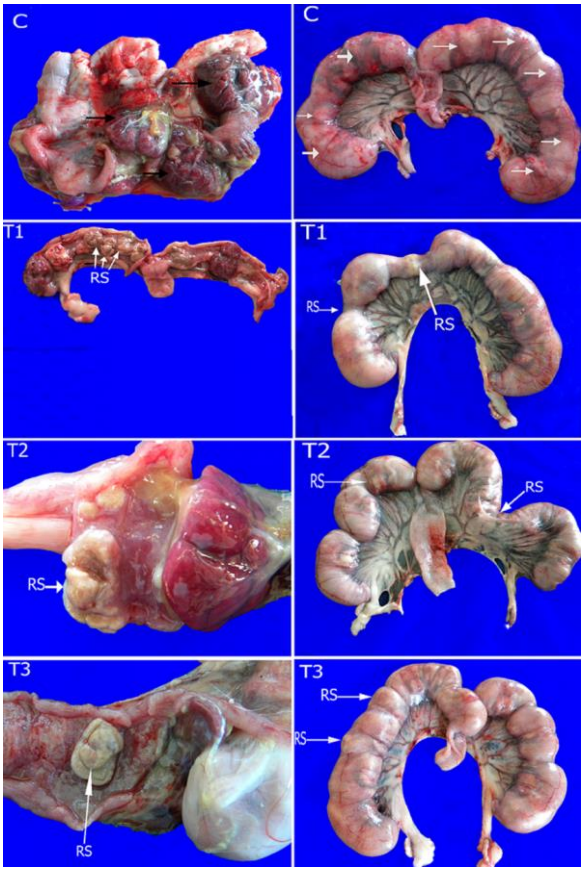


**Fig. 9:** A photograph of the uteri of control (C) and khat treated dams: low dose (T1), medium dose (T2), high dose (T3), showing live embryos (arrows) inside the uteri rabbit at 28th day of gestation), resorbed fetus (RS).

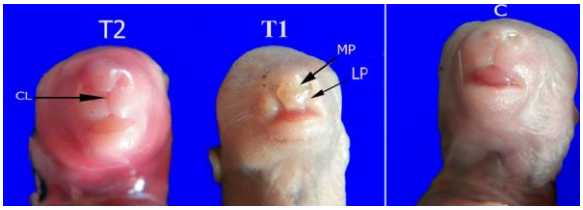


**Fig.** 10**:** A photograph of control fetus (C)and feti of khat treated dams: low dose (T1) , medium dose (T2) showing incomplete fusion of the lateral process (LP) and medial nasal process (MP), cleft lip (CL).

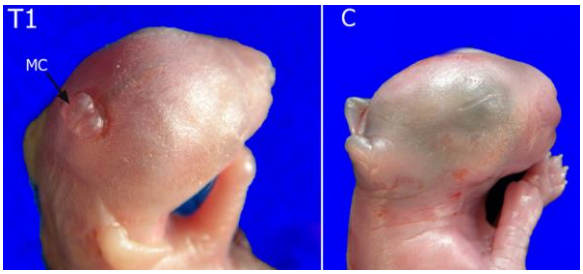


**Fig.** 11**:** A photograph of control fetus (C)and fetus of khat treated dams: low dose (T1) showing meningeoceale (MC).

The subcutaneous hemorrhage and congestion all over the fetal body did not recorded by Isalm *et al.*, 1994 in rats. This hemorrhage may be due to that the khat increase the fluidity of blood or it may increase capillary fragility (Mwenda, *et al.*, 1988) in human.

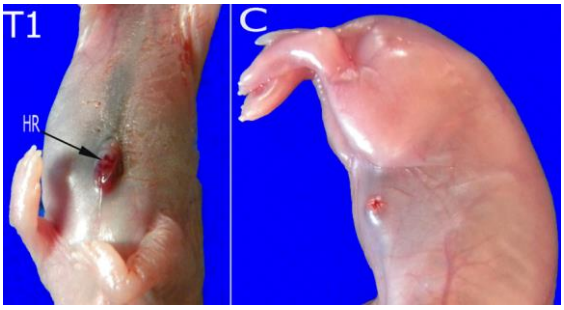


**Fig.** 12**:** A photograph of control fetus (C)and fetus of khat treated dams: low dose (T1) showing umbilical hernia(HR).

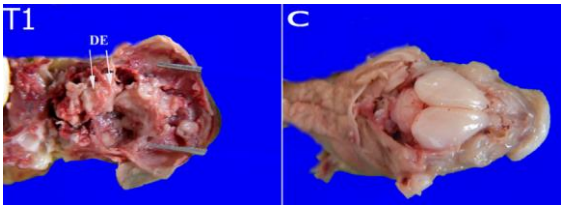


**Fig.** 13**:** A photograph of dorsally opened rabbit fetus cranium: control (C) and low dose (T1) showing complete degeneration of the brain (DE).

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