Research Paper

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TERATOGENIC EFFECT OF THE WATER EXTRACT OF BITTER GOURD (*MOMORDICA CHARANTIA*) ON THE SPRAGUE DAWLEY RATS

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Abstract

It has been reported that the water extract of the whole unripe fruit of Momordica charantia can significantly reduce blood glucose levels. However the safety of its use during pregnancy has not been fully investigated. The aim of this investigation is to determine the safety of this extract during pregnancy. The water extract of the unripe fruit was given to pregnant Sprague Dawley rats on days 7, 8, 9, 10, 11, 12, 13 and 14 of gestation. The litter size was determined for each group and the litters were examined for gross malformations. The gross and histological examinations of various organs of the litters were also carried out. Results show that 8.65% of the litters from experimental animals were malformed as against 1.62% of control. It also showed that 31.2% of all the malformed litters had multiple congenital malformations. It also showed that the experimental rats had nine resorption sites while control had none. This demonstrates that the water extract of Momordica charantia is teratogenic in Sprague Dawley rats and should be used with caution in man.

Key words: Momordica charantia teratogenicity

Introduction

The placenta provides a selective barrier between the fetus and the mother. This barrier protects the fetus from some harmful substances crossing from the mother to the fetus. Chemicals (including drugs) are among some harmful agents that sometimes breach this barrier.

Since teratogenicity of thalidomide was highlighted a lot of research has been undertaken to determine the safety of different drugs during pregnancy. To emphasize the importance of this, most drug manufacturers always include the caution “not to be used in pregnancy unless prescribed by a physician”, in their directions for use of drugs.

Herbal preparations have been in use before the onset of pharmaceutical products. However, their use has been dramatically increased recently; hence such terms as “herbal medicine and alternative medicine” are gradually finding their way into medicinal curricula. The use of herbs for the management of different illnesses is appealing because it is cheap and readily available. It also reduces the stress of the hospital environment. Herbalists have been practising their trade for ages. This is usually done with some degree of secrecy. Furthermore, the complications that might be associated with their use are not usually highlighted.

Extracts from different parts of Momordica charantia have been reported to have medicinal values. Different extracts have been reported used for the treatment of infections (Chen et al., 2009, Sonibare et al., 2009),

hyperglycemia (Uche-Nwachi and Mitchell, 2004; Han et al., 2008; Shih et al., 2009; Gbolade, 2009) and wound healing (Teoh et al., 2009; Alam et al., 2009; Lii et al., 2009; Ono et al., 2009).

One of the commonest uses is in the control of blood glucose in diabetics. The mode of action in the hypoglycemic property of *Momordica charantia*, has been extensively studied with varying results. Krawinkel and Keding (2006), reported that, the mechanism of action, whether it is via regulation of insulin release or altered glucose metabolism and its insulin-like effect is still under debate while Shih et al. (2009), reported that Momordica charantia extract acts by decreasing insulin resistance and increases glucose transporter protein 4 (GLUT4) in skeletal muscles. Herbalists usually administer the water extracts to diabetics to control their blood glucose.

The incidence of diabetes has been on the increase. Many diabetic patients in rural communities use herbal hypoglycemic agents for the control of their blood sugar. One of the herbs most frequently used is coralli/bitter gourd (*Momordica charantia*). *Momordica charantia* has been native to the tropics. According to Seaforth (1998), the herb is found in some parts of the Amazon, East Africa, Asia, and the Caribbean and has been used as a folklore medicine to treat various ailments including diabetes. Several investigators including Welinhinda et al. (1986), Ahmed et al. (1998), Ahmad et al, (1999), Sitasawad et al (2000), Lin et al. (2001), Miura et al. (2001), Vikrant et al (2001), Biyani et al, (2003) and Uche-Nwachi and Mitchell (2004), Gbolade et al (2009), Shih et al (2009), Han et al (2008), also had reported that the hypoglycemic property of the aqueous extract of Momordica charantia. Singh et al. (1989), Day et al (1990), Higashino et al (1992), and Ali et al (1993) had reported that extracts of the entire unripe fruit have been used as a hypoglycemic agents. However, other investigators, including Chongkol et al (1987), Karuanayake et al, (1990), Cakici et al (1994), and Platel and Sirnivasan (1995), reported that the hypoglycemic property of *Momordica charantia* depended on what part of the fruit used and the method of extraction employed. Uche-Nwachi and Mitchell (2004) reported that, while the unripe whole fruit extract had comparable hypoglycemic property to insulin and oral hypoglycemic drugs, that the ripe fruit did not possess this property. They also found that the extract lost its hypoglycemic property if not refrigerate. In spite of findings of these investigators, not much has been done to determine the safety of the use of *Momordica charantia* extracts in pregnancy. The aim of this investigation is to determine its possible teratogenic effect and its safety in pregnancy.

Materials and Methods

Animals

Fifty three Sprague Dawley female rats weighing 280-380 grams were selected from the Animal House in the Faculty of Medical Sciences, University of the West Indies. The animals were acclimatized for two weeks, under standard conditions of temperature and illumination (12 hr light and 12 hrs dark) cycle. Animals had access to food (standard rat diet) and water *ad libitum*, and were cared for according to the Animal House Committee regulations on "the Care of Experimental Animals".

Confirmation of pregnancy

Vaginal smears were taken every two hours during the day, to determine the estrus cycle based upon vaginal cytology. Rats with proestrus vaginal cytology were grouped in threes. Each group was placed in a cage with a virile male for mating. Mating was confirmed by the presence of spermatozoa in the vaginal smear. This was the ***sperm positive date*** and corresponds to day zero in the dating of the animals. The pregnant rats were weighed daily. A significant weight gain on the 10th day of gestation was also confirmatory of pregnancy.

Preparation of Extracts

Unripe *Momordica charantia* was obtained from the local market. The water extract was prepared using 5kg of the whole unripe fruit including the seeds and 300ml of distilled water. This was blended using a commercial blender until pulp was formed. This was then strained using a cheese cloth to remove the coarse fibers.

Treatment

The experimental animals were divided into groups of three. A total of 8 groups (1-8) were acclimatized in separate cages before they were mated with virile males at different days in gestation. The rats in group 1 cage were commenced on the extract on the 7^{th} day of gestation, while the animals on cage 8 were commenced on the 14^{th} day of gestation. The remaining cages (2-7), were started on successive days of gestation. Using the dating method, two

milliliters of the extract was administered orally from days 7, 8, 9, 10,11,12,13 and 14, which corresponded to cages 1-8 of gestation respectively (Uche-Nwachi et al., 2004).

Control groups made of 29 female rats were divided into groups of 4.Each group was mated with a viral male. This control group received distilled water orally in place of the extract. The aim was to get the same numbers of pups from the control and experimental groups for statistical analysis. Rats with no significant weight gain were sacrificed on day 22 to observe any resorption sites. After delivery the litter size and average pup weight from each group (control and experimental) was recorded. Malformed pups were also recorded. Pups were weighed daily and were individually marked when weaned. Pups were allowed to mature up to 45 days of age, when they were sacrificed via ether asphyxiation (Miettinen et al., 2002). Important organs which will determine the viability of the pups (brain, heart, lungs, liver, spleen) and organs from the same embryonic origin (kidney, ovaries, and testis), were harvested and observed for the presence of defects. The organs were weighed, and then fixed in Bouin's fluid. Paraffin sections, (0.5µ thick) were cut and stained with hematoxylin and eosin. A one-way ANOVA was used for the statistical analysis of the organ weight differences between experimental and control pups.

Results

Birth weights

Results showed that the mean birth weight of the experimental pups was 6.34g vs. 7.06g ($p < 0.05$), and that 16 of the pups from the experimental animals (8.65%) were malformed as against 3 from the control (1.62%) {Table1}. These result also showed that while 5 pups from the experimental groups had multiple congenital malformations, none from the control group had such malformations (Table1).The result further showed that while the control rats had no resorption sites, the experimental rats had 9 resorption sites (Table 1, Figure 4a).

Table 1: Mean litter size, body weight, resorption sites and congenital malformations in the pups from control and experimental animals

	Control	Experimental (MC)
Dams	29	24
Total number of pups	185	185
Males	83	83
Females	102	102
Mean litter size Mean birth weight	6.4 7.06 g	7.7 6.34 g
Gross congenital malformation	3	16
% of congenital malformation	1.62	8.65
Males with malformations	1	10
Females with malformations	2	6
Multiple malformations	0	5
Resorption sites	0	9

Malformations

The result also showed that while only 1.62% of the control pups were malformed, 14.8% of the pups from day 7, 14.3% from day 8, 7.4% from day 9, 12.2% from day 11, 2.9% from day 12, 21.4% from day 13 had malformations. It also showed that there were no malformations on days 10 and 14 (Table 2).

Mean organ weight

The result also showed that the mean weights of the kidney, the liver, the spleen, and the brain were significantly lower in the experimental pups, while the heart was significantly increased when compared with control ($p<0.05$) {Table 3}.

Fig 1a

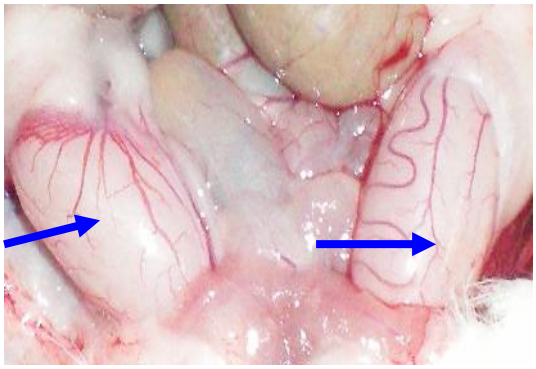

Fig 1b

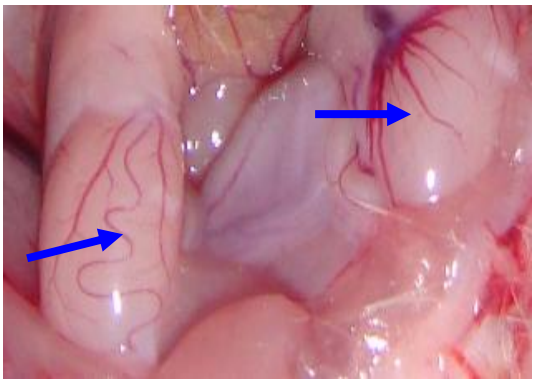

Fig 1c

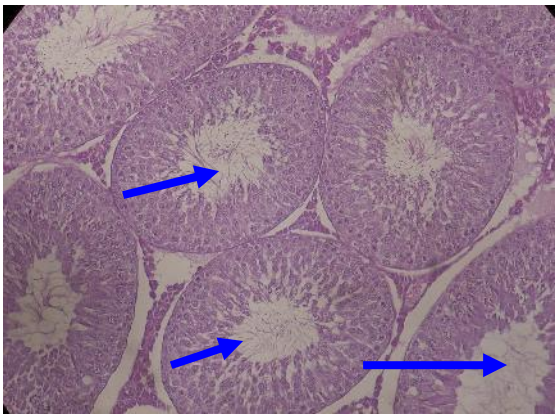

Fig 1d

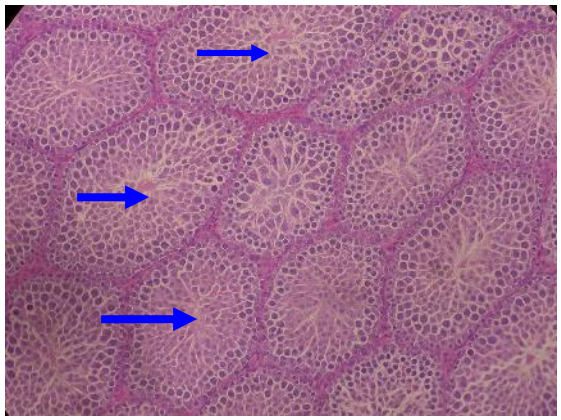

Figure 1a: Photograph of normal testes from control rats x40. Note the size and shapes of the testes (arrows).
Figure 1b: Photograph of undescended testes, (arrows) x40. Note the size and position of the testes inside the abdominal cavity.
Figure 1c: Photomicrograph (H&E), of normal testis from control rats x40. Note the canalization of the seminiferous tubules (arrows).
Figure 1d: Photomicrograph (H&E), of the undescended testis from experimental rats.x40. Note the absence of canalization of the seminiferous tubules (arrows)

Histology

Histological analysis also showed that the undescended testes had uncannalized seminiferous tubules (Fig 1d), and that the distended uterine horns had wide lumina (Figure 2c). Further analysis also showed that the atrophic ovary had no viable follicles (Fig 3b). It also showed multiple resorption sites in the experimental animals (Figure 4a).

Discussion

According to, Chan et al (1984), Leung and Yeung, (1987), Aguwa and Mittal. (1983), Grover and Yadav (2004, *Momordica charantia* extracts have abortifacient properties. It has also been reported to reduce fertility in both males and females by Farnsworth and Waller (1982), and Basch et al. (2003). This present study showed that, there were 9 resorption sites in the experimental animals while the control had none (Table 1). The present study also

showed that 8.7 % of the experimental pups had malformations while only 1.6% of the control had malformations (Table 1).

Table 2: Distribution of malformations and resorption sites among the different days in gestation, the extracts was initiated

Day of gestation	Litter Size	Normal Pups	Pups Affected	Malformation types	Malformation %	Resorption Sites
Control	185	183	Female Female Male	Distended uterine horn and uterine atrophy Ovarian atrophy, Unilateral testicular agenesis	1.62	0
Day 7	27	23	Female Female Female Male	Unilateral ovarian hypertrophy, Bilateral ovarian atrophy Bilateral ovarian atrophy, enlarged left lung and splenomegaly Cryptorchidism	14.8	0
Day 8	7	6	Female	Uterine horn atrophy	14.3	9
Day 9	27	25	Male Male	Bilateral testicular atrophy and hepatomegaly Hepatomegaly	7.4	0
Day 10	14	14	0		0	0
Day 11	41	36	Male Male Male Male	Cryptorchidism Cryptorchidism and splenomegaly Bilateral testicular atrophy, Hepatic atrophy and renal hypertrophy, Bilateral testicular atrophy and splenomegaly, Anencephaly and spinabifida	12.2	0
Day 12	34	33	Male	Unilateral testicular hypertrophy	2.9	0
Day 13	14	11	Female Female Male	Distended uterine horns Distended uterine horns Hepatic atrophy	21.4	0
Day 14	22	22	0		0	0

Table 3: Significant organ weight difference (P< 0.05)

Day	Organ	Mean organ weight of control (g)	Mean organ weight experimental (g)	P value Significant at P<0.05
8	Brain	1.88	1.77	0.002
9	Heart	0.78	0.87	0.010
10	Heart	0.75	0.84	0.042
11	Liver	10.65	8.69	0.001
12	Liver	11.07	9.54	0.004
13	Brain	1.90	1.82	0.020
14	Kidney	1.89	1.59	0.003
14	Lungs	1.23	1.05	0.008
14	Liver	10.00	8.53	0.019
14	Spleen	0.82	0.66	0.038

Fig 2 a

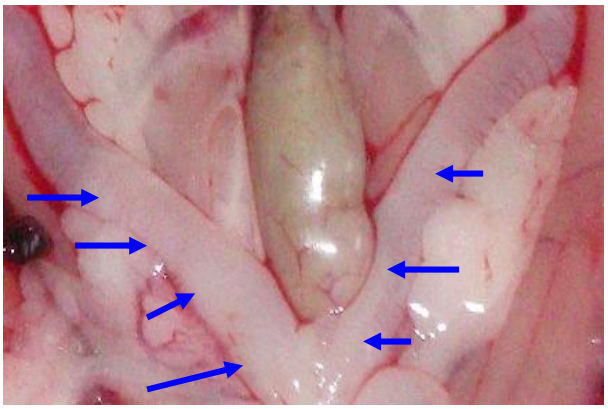

Fig 2b

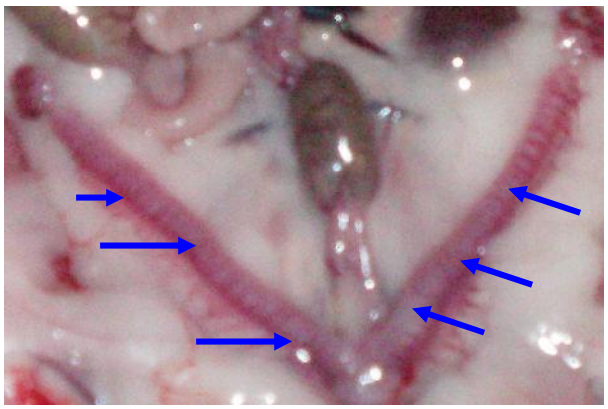

Fig 2c

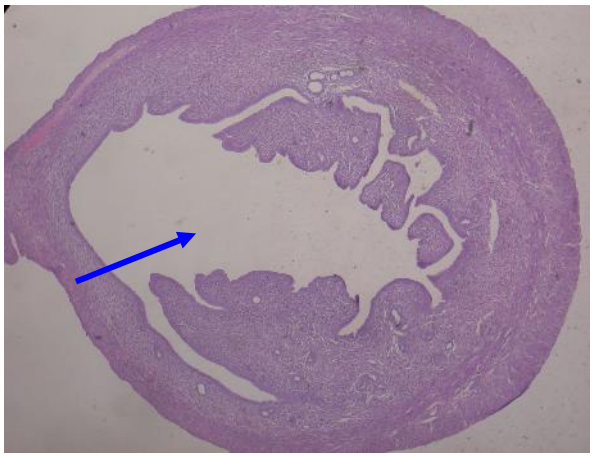

Fig 2d

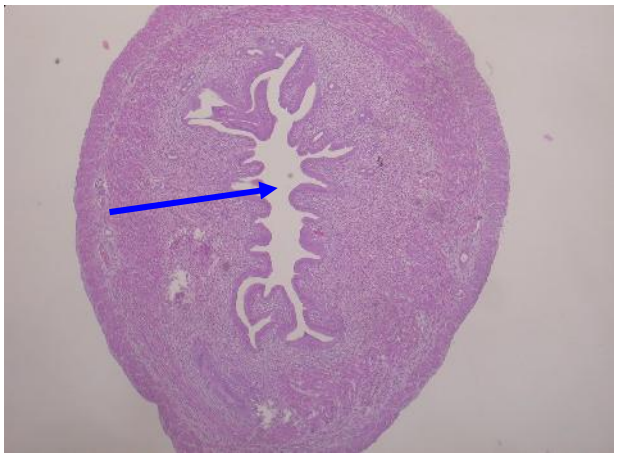

Figure 2a: Photograph of distended uterine horns from experimental rats (arrows) x40.
Figure 2b: Photograph of normal uterine horns from control rats (arrows) x40.
Figure 2c: Photomicrograph (H&E), of a cross section of distended uterine horn from experimental rat x40. Note the luminal size (arrow)
Figure 2d: Photomicrograph of a cross section of a normal uterine horn from control rat, x40. Note the size of the lumen (arrow), when compared with Fig 2c above.

Fig 3a

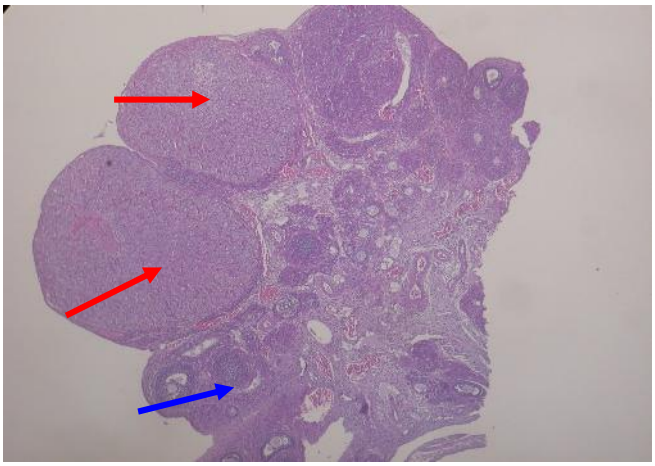

Fig 3b

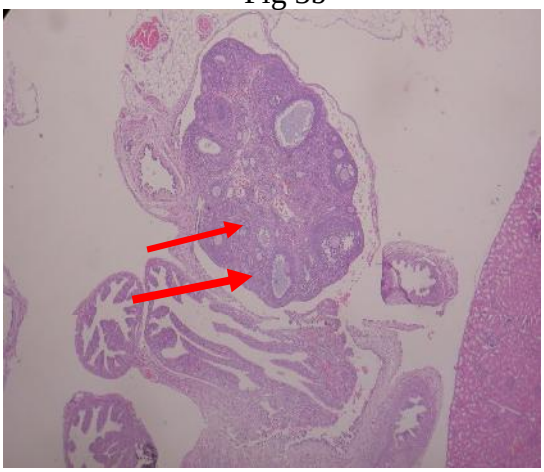

Figure 3a: Photomicrograph (H&E), of a normal ovary from control rat, x40. Note the presence of corpora lutea (red arrows), and developing follicles (blue arrow).
Figure 3b: Photomicrograph (H&E), of the ovary from experimental rat x 40. Note the absence of corpus luteum and the absence of viable follicles.
Figure 4a: Photograph of resorption sites in the uterine horns of experimental rats (arrows) x 40.
Figure 4b: Photomicrograph (H&E), of early resorption from the uterine horn of experimental rat x40. Note the cavity of the horn filled with resorbed debris (arrow).

It was also demonstrated in this study that, the teratogenicity of the water extract of *Momordica charantia* was dependent on the day of gestation it was administered. The present study also showed that reproductive organs were affected most in the malformed liters (Table 2), and that administering the extract from day 7, 11, and 13, showed the greatest number of malformations (Table 2). Furthermore, our result also showed that there was significant reduction in the weights of the brain, liver, kidney, lung, and spleen in the pups from the experimental animals, while there was a significant increase in the weight of the heart (Table 3). This implies that most organs from the pups of the experimental animals were affected in one way or another. The alterations in morphology and weight obviously may affect the respective functions of the affected organs.

We therefore conclude that the water extract from the unripe fruit of *Momordica charantia* is teratogenic and that this was dependent on the period in gestation it was administered, and that the reproductive organs of the pups were most affected. We therefore advise that this preparation should be used with utmost caution during pregnancy in man.

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Fig 4 a

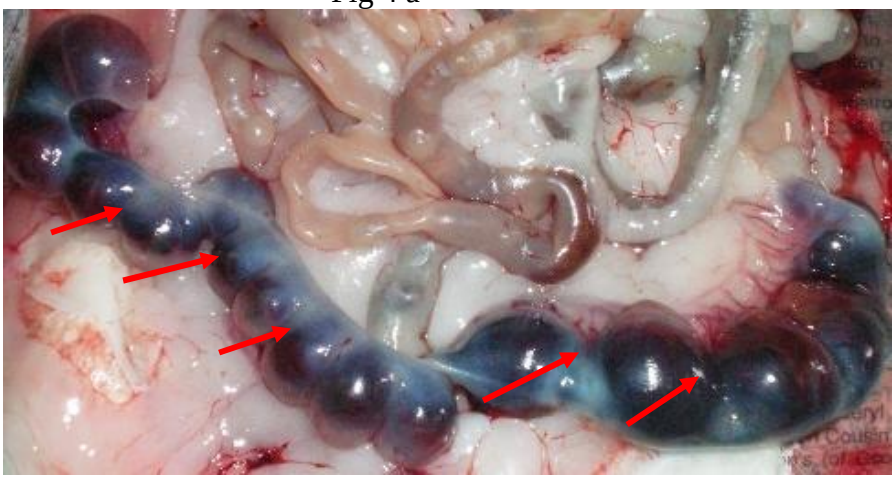

Fig 4b

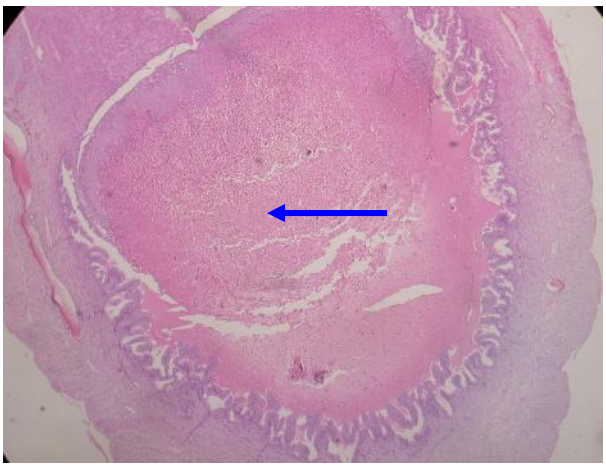

Fig 4c

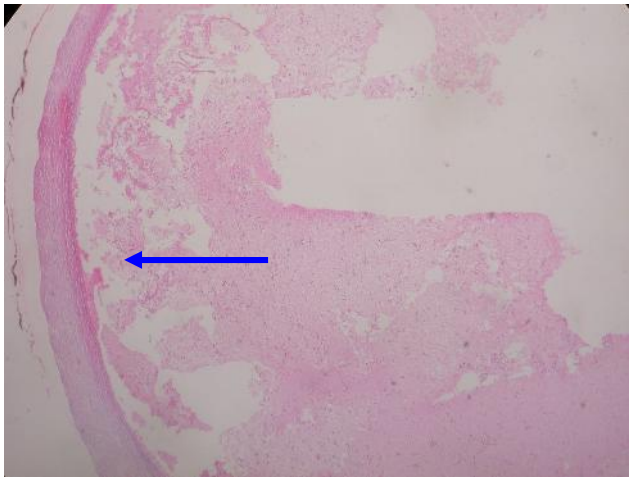

Figure 4c: Photomicrograph (H&E), of late resorption site from the uterine horn x40. Note the re-epithelization of the endometrium (arrow).

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