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# The Impact of Proximol (*Cymbopogon proximus*) Intake on Pregnant Albino Rats and their Fetuses During Gestation Period

## Impacto de la Ingesta de Proximol (*Cymbopogon proximus*) en las Ratas Albino Embarazadas y sus Fetos durante el Período de Gestación

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**SUMMARY:** Halphabarol, the active principle of Proximol, is the most potent of the four antispasmodics present in the national desert weed *Cymbopogon proximus* or "Halfa Bar". Halphabarol is of great value for the management of renal colic and in the expulsion of ureteric calculi as it causes dilation of the ureter below the site of calculus while active propulsion is maintained. Evaluation the congenital malformation of proximol in pregnant albino rats during gestation period. The virgin female rats were mated with male rats and the pregnant rats were orally administered a human equivalent dose (0.05 mg/kg) of Proximol from $5^{th}$-$20^{th}$ gestation day. At day 20 of pregnancy, all rats were anesthetized to obtained maternal and fetal data. The treatment group displayed some disorders, which can be summarized as growth retardation, external anomalies, embryonic resorption, and skeletal malformation. We concluded that the oral administration of Proximol resulted in embryonic abnormalities and skeletal malformations.

**KEY WORDS: *Cymbopogon proximus*; Halfa Bar; Malformation.**

## INTRODUCTION

Herbal medicines have a long history of use for the prevention and treatment of diseases; their use can be traced back to the first written certificates of humanity, through antiquity, middle ages, and even modern times (Williamson, 2003). Often for efficient and documented herbal medicinal products, the toxicity can be relatively undetected. Unlike conventional drugs, the toxicity of traditional herbal medicines is not often evaluated (Suter, 2006; Smart *et al*., 2011). However, the majority of the population does not seem alarmed by this lack of testing since they believe that because these products have been used thus far, they should be free from toxicity (Luyckx & Naicker, 2008).

The active ingredients in medicinal herbal plants must readily transit to the placenta, accumulating at a high enough dose to cause risk or harm to the fetus during pregnancy and be present at a specific time during gestation to exert its effect on the developing fetus (Saad *et al*., 2006; Wilson, 2007).

The genus *Cymbopogon proximus* of the family Gramineae, locally known as Halfa-bar, is an aromatic, densely tufted grass growing wildly and widely in Upper Egypt. The Halfa-bar, a conventionally used medicinal herb claimed to be effective against renal spasms, lacks appropriate toxicological testing. The herb exerts its unique pharmacological action through relaxation of the smooth muscle fibers without inhibiting the propulsive movement of the tissue; thus, it is traditionally used in the expulsion of renal and ureteric calculi (Evans *et al*., 1982).

The use of medicinal plant products to treat various ailments is a common practice in many developing countries. However, a lack of information on the adverse effects of these plants raises questions on their safety and possible adverse side effects. This study was undertaken to evaluate the potential toxic effects of *Cymbopogon proximus*, Halfa-bar on pregnant rats and fetal development. The results indicate some teratogenic effects of Proximol (halphabarol) on the pregnant albino rats during the gestation period.

## MATERIAL AND METHOD

**Drug Used.** Proximol tablets were purchased from Kahira Pharmaceuticals, Cairo, Egypt. Each tablet contains 0.4 mg halphabarol as a main active ingredient.

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**Animal Housing.** Adult male and virgin female albino rats, *Rattus norvegicus* (170-180 g), bred in the animal facilities of our own Zoology department were used. This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Cairo (CUFS/F/46/14). All scarification was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering. Two female rats were selected and caged together with one male rat overnight under controlled environmental condition of temperature (25 ± 2 ºC), humidity (60 ± 20 %), and light (12 light-12 dark cycles). The first day of gestation was determined by the presence of sperm in the vaginal smear (McClain & Becker, 1975).

**Experimental Design.** Pregnant rats were divided into two groups (n = 12 per group). The control group received saline. The experimental group received 0.05 mg/kg of Proximal diluted in saline. The dosing regimen was based on human equivalent dose (HED). Treatments were administered orally by gavage from gestation day (GD) 5 up to 20, defined as the period of structural development the embryonic stage for rats (WHO, 2001). At the 20$^{th}$ GD, each dam was submitted to a cesarean section. The animals were anesthetized with sodium pentobarbital (100 mg, i.p.). The abdomen was incised; the gravid uteri of the pregnant rats were removed. Investigations were carried on mothers, collected uteri and fetuses as follow:

- The toxicity in the treated dams was determined by observing external symptoms, maternal mortality and changes in body weight. The weights of the pregnant rats were recorded at the 5$^{th}$ and 20$^{th}$ GD and the % of change in maternal weight through the gestation was calculated as % change in maternal weight = (wt. of 20th day- wt. of 1st day / wt. of 20$^{th}$ day) x 100.

- Gravid uterus was removed and weighed with its contents, and total number of implantation sites and living, resorbed, or still-birth fetuses were recorded to calculate fetal mortality rate and the post-implantation loss index, which was calculated according to (Christian, 2001).

- Post-implantation loss index = No. of implantation sites- No. of live fetuses/ No. of implantation sites x 100.

- The fetuses were examined for external malformations; their body weight and length were recorded.

- Fetal skeletal examination: Examination to skeletal components, control and treated fetuses were skinned and eviscerated; then, they were fixed in 95 % ethyl alcohol for 10 days. Subsequently, they were cleared in acetone solution for 7–10 days. After that fetuses were stained in alcian blue and alizarin red S stains (alcian blue, alizarin red S, glacial acetic acid, 70 % ethyl alcohol) for 3 days at 37 ºC. After staining, the specimens were transferred into an aqueous solution of 1 % KOH for maceration. So, specimens were transferred to pure glycerol via ascending series of glycerol–KOH and stored in pure fresh glycerol. The cartilages attain blue color while the bones elements appeared red. Finally, under dissecting microscope, the stained skeletons were examined.

**Statistical analysis.** All the values are presented as means (m) ± standard error of the mean (S.E.M). Comparison between two different groups was carried out using the independent student T-test.

## RESULTS

### Morphological studies

**Pregnant rat's toxicity.** The pregnant rats treated with Proximol orally during the gestational period (5$^{th}$ - 20$^{th}$ day) showed no external signs of toxicity. No mortality cases were recorded. The average maternal body weight was recorded for the two groups on the 5$^{th}$ and the 20$^{th}$ day of gestation (Table I).

The uteri of the control pregnant rats on GD 20 had a normal distribution of implanted fetuses between the two horns (Fig 1A). The uteri of the treated pregnant rats showed asymmetrical distribution of fetuses in the two uteri tubes and a reduced number of fetuses. The uterine tubes showed clearly visible embryonic resorbed sites (Fig 1B).

**Effect of Proximol on fetuses.** The morphological examination of the fetuses revealed that Proximol caused growth retardation, represented by a decrease in fetal body weight and body length (Table I). There was a significant ($P \leq 0.05$) reduction in fetus weight in the treated group

Table I. Effect of Proximol on fetus weight, fetus length, and mother weight loss at the 20$^{th}$ day of gestation.

| Group | Fetus weight (F.WT) | Fetus length (F.L) | Mother weight loss (M.WT) |
|---|---|---|---|
| Control (A) | 2.25 ± 0.17 | 5.61 ± 0.12 | 53.42 ± 9.43 |
| Treatment: 0.05 mg/kg (B) | 3.31 ± 0.12$^{a}$ | 5.38 ± 0.06 | 35.0 ± 3.0 |

Values are expressed as means± standard error of the mean (S.E.M). The statistical differences were analyzed by independent samples T-test. a = $P \leq 0.05$ compared with control.

compared to that of the control group. The shape, weight, and length of the fetus from control animals appeared normal (Fig 2A). The fetus of mother treated with Proximol showed various morphological anomalies such as hematoma, clubfoot, missing ear, and loss of hand digits (Table II; Figs. 2B-D).

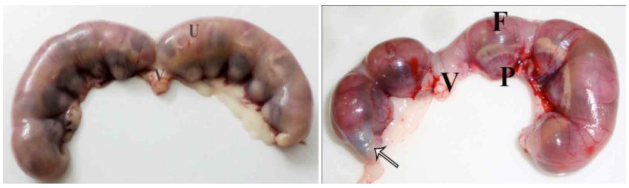


Fig 1. Photographs of the uterus of pregnant rats at the 20th day of gestation. A) Control. Showing normal symmetrical distribution of fetuses in the two uterine horns. B) Treated. Asymmetrical distribution of fetuses in the two uterine horns. Left uterine horn showing visible embryonic resorption sites (arrow).
F=Fetus, P=Placenta, V=Vagina, Ut=Uterus

The administration of therapeutic doses of Proximal (0.05 mg/kg, p.o.) during the gestational period induced skeletal abnormalities; the skeleton malformations in all groups are represented in Figs. 3-8.

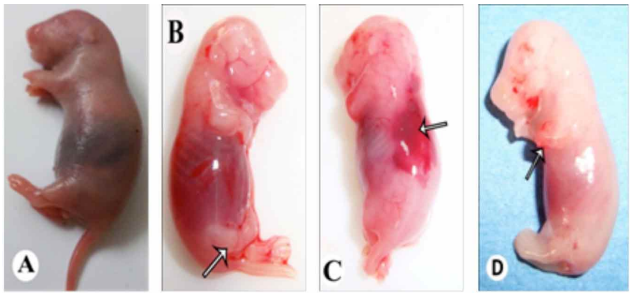


Fig 2. Photographs of fetuses at the 20th day of gestation. A) Control. Fetus exhibited normal morphology and normal length. B-D) Treated. Showing: clubfoot (arrow, B), hematoma at the back (arrow, C), and fore limb without digits (arrow, D).

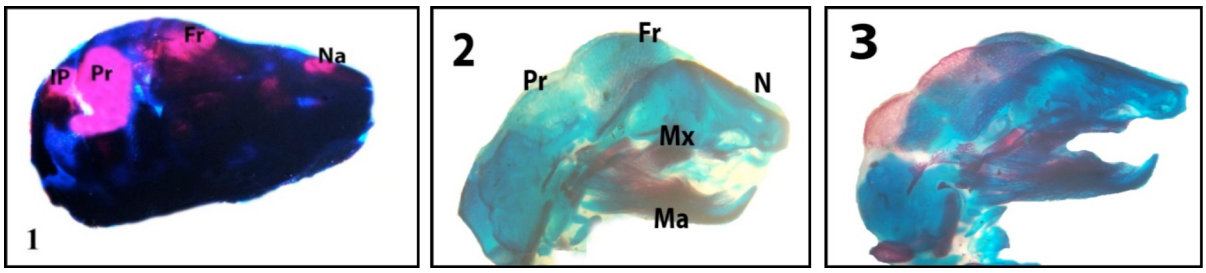


Fig 3. Photographs of the cranial skeleton of fetuses at the 20th day of gestation. (Alcian blue & Alizarin red stain). 1) The cranial skeleton of the control fetuses showing complete ossification of the cranial bones. 2 & 3) The cranial skeleton of fetuses maternally treated with Proximol showing unossified cranial bones (2) and incomplete ossification of all cranial bones (3). Na = nasal, Mx = maxilla, Ma = mandible, Fr = frontal, P = parietal and IP = interparietal.

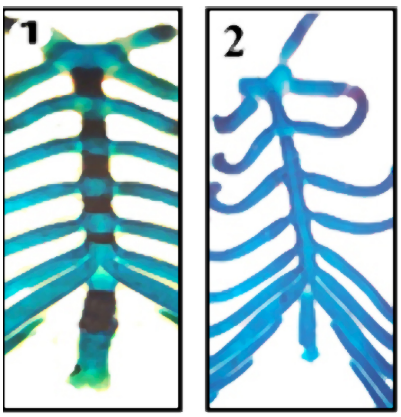


Fig 4. Photographs of the sternum of fetuses at the 20th day of gestation. (Alcian blue & Alizarin red stain).1) The sternum of control fetuses showing complete ossification of the sternbrae bones. 2) The sternum of fetuses maternally treated with Proximol showing unossified sternbrae bones.

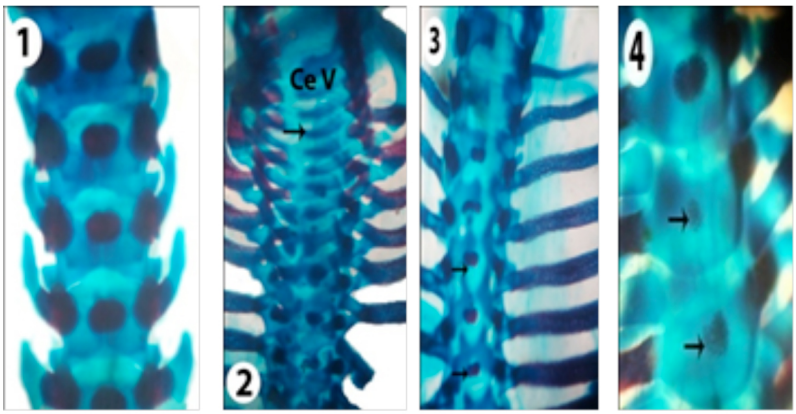


Fig 5. Photographs of the vertebral column of fetuses at the 20th day of gestation. (Alcian blue & Alizarin red stain). 1) The vertebral column of control fetuses showing complete ossification of all vertebrae. 2-4) The vertebral column of fetuses maternally treated with Proximol showing unossified centers of cervical vertebrae (2), dumbbell-shaped thoracic vertebrae (3), and less ossified centra (4). Ce V = cervical vertebrae.

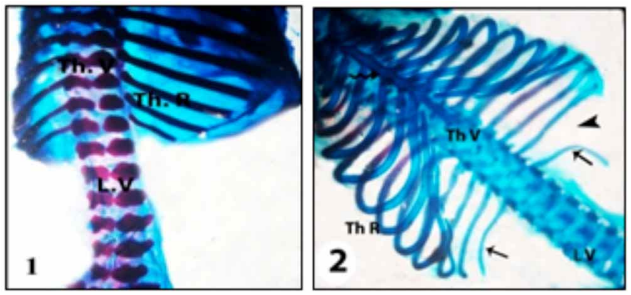


Fig. 6. Photographs of the ribs of fetuses at the 20th day of gestation (Alcian blue & Alizarin red stain).1) The ribs of control fetuses showing complete ossification and normal shape of the ribs. 2) The ribs of fetuses maternally treated with Proximol showing curved ribs (arrow), costal separation (head arrow), and incomplete ossification of ribs. Th V = thoracic vertebrae, Th R = thoracic ribs and LV = lumbar vertebrae.

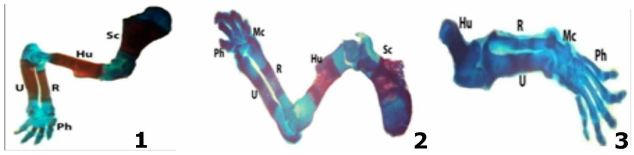


Fig. 7. Photographs of the pectoral girdle and fore limb of fetuses at the 20th day of gestation. (Alcian blue & Alizarin red stain). 1) The pectoral girdle and fore limb of control fetuses showing complete ossification of all bones. 2 & 3) The pectoral girdle and fore limb of fetuses maternally treated with Proximal showing incomplete ossification of all bones (2) and unossified MC and lack of ossification of radius and ulna (3). Sc = scapula, Hu = humerus, R = radius, U = ulna, MC = metacarpals and Ph = phalanges.

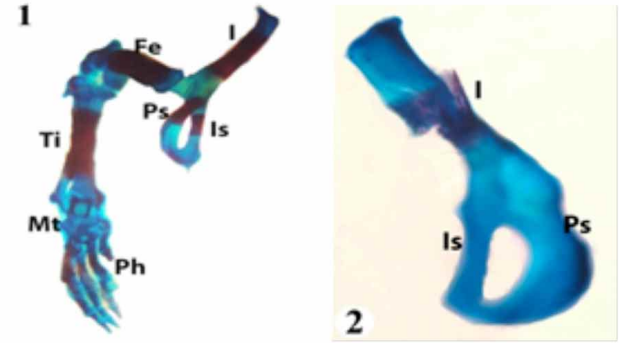


Fig 8. Photographs of the pelvic girdle and hind limb of fetuses at the 20th day of gestation. (Alcian blue & Alizarin red stain). 1) The pelvic girdle of control fetuses showing complete ossification of all bones. 2) The pelvic girdle of fetuses maternally treated with Proximol showing incomplete ossification of ilium and unossifed ischium and pubis. I = ilium, Is = ischium, P = pubis, Fe = femur, Fi = fibula, Ti = tibia, MT = metatarsals and Ph = phalanges.

In general, the skeleton of the rat fetus GD 20 consists of two main parts, the axial and the appendicular skeleton. The axial skeleton contains the bones of the skull (nasal, frontal, parietal, maxilla, and mandible), vertebral column (cervical, thoracic, lumbar, sacral, and caudal vertebrae), ribs, and sternum. The appendicular skeleton comprises the bones of the pectoral girdle (scapula, clavicle), pelvic girdle (ilium, ischium, and pubis), fore limbs (humerus, radius-ulna, and metacarpals), and hind limbs (femur, tibia-fibula, and metatarsus). All the bones are present in well-ossified condition, stained red in all fetuses, and of correct size and shape. Fetuses from the treated mothers showed some aberrations, manifested as incomplete ossification of skull bones. The abnormalities in both vertebrae and vertebral central were manifested as incomplete ossification, missing central discs and lack ossification. Rib abnormalities included irregularly shaped ribs and incomplete ossification. Fore limbs showed incomplete ossification of the humerus, radio-ulna, and metacarpal. In addition, the bones of the pelvic girdle showed incomplete ossification of ileum and unossification of ischium and pubis.

Table II. Effect of Proximol on fetus morphology (gross pathological) at the 20th day of gestation.

| Groups | No.of examined fetuses | Hematoma | Hid limbs anomalies | Fore limbs anomalies | Digits | Deformed ear |
|---|---|---|---|---|---|---|
| Control A | 82 | 0 | 0 | 0 | 0 | 0 |
| Treated B | 74 | 3 | 4 | 2 | 4 | 6 |

## DISCUSSION

Nowadays, herbal medicines are gaining popularity owing to the decreasing efficacy of the modern medicines. Furthermore, many synthetic drugs cause baleful side effects. There is an absence of reliable embryo protective drugs to curtail the teratogenicity of modern medicine; herbal drugs could fulfill this need and are being evaluated for their

embryo protective effect (Shingadia & Dalvie, 2014). Herbal drugs are very primitive in comparison with the chemical drugs commonly used today. Nonetheless, herbal medication is a common treatment method for many diseases health issues in the developing countries (Kamboj, 2000).

Recently many reports have indicated a wide use of medicinal herbs by pregnant women (Hepner *et al*., 2002). During pregnancy, several agents can interfere with the normal development of the embryo, which leads to functional and/or morphological defects. The potential of the teratogenic effects depends on many factors, including the maternal-fetal genotype, the stage of embryonic development, the time of exposure to the substance (Spritzer *et al*., 2001), and the mutagenic events, such as chromosomal aberrations and non-disjunction (Oliveira *et al*., 2009).

In our study, orally treating pregnant rats with Proximol during fetal organogenesis caused a decrease in the weight of the mother. However, no mortality cases were observed. Embryonic growth retardation was observed and represented by a decrease in fetal weight and length, and an increase in resorbed sites and post-implantation sites. Furthermore, the administration caused some developmental disorders including deformed limbs and ears, hematoma, and skeletal malformation, including delayed ossification. The reduction in maternal weight may be due to a decrease in diet and water intake or because the drug caused resorption and growth retardation. The limb deformations, such as clubfoot, may be caused by the direct action of the drug components and its metabolites on the fetal tissue, or indirect effects by altering the physiological state of the mother. Skeletal staining revealed incomplete ossification of the skull, limb bones, and sternbrae, and wavy ribs and dumbbell shaped or absent vertebral centra. Weak ossification of the fetal skeleton may be a reason for fetal growth retardation and the consequential decrease in fetal weight. Delayed ossification of several bones may be a result of alternations in calcium metabolism or deviations in calcitonin levels in the developing fetus, leading to weak bone development.

Teratogenesis may be viewed as differential growth disturbances of some tissues, resulting in the failure of the fetus to maintain its normal expected growth potential for any of the gestational periods (United Nations, 1977).

The phytochemical constituents found in the aqueous extract of Halfa-bar, the common name of *Cymbopogon proximus*, are terpenes, tannins, flavonoids, saponins, alkaloids, carbohydrate or glycosides, and phenolic glycosides (Ibrahim & El-Khateeb, 2013). Several studies have analyzed these components, which are also found in other plants, including non-herbal plants.

In animal studies, phenols have been reported to be embryotoxic and fetotoxic, but not teratogenic. Furthermore, phenols generally do not caused developmental defects except at doses that cause maternal toxicity. Extra caution is exercised regarding the exposure of pregnant women. Since phenols have been shown to be genotoxic at high doses, medical counseling is recommended for acutely exposed pregnant woman. There are no known teratogenic effects from phenol acute exposure (ATSDR, 2016).

The effects of high concentration of phenols are lethal and low concentrations are teratogenic to Bufo arenarum embryos and the young tadpoles of this native species (Paisio *et al*., 2009).

The secondary component, tannins, has antioxidative property but also exert other physiological effects, such as accelerating blood clotting, reducing blood pressure, decreasing the serum lipid level, producing liver necrosis, and modulating immunoresponses (Chung *et al*., 1998). Saponins are secondary metabolites of glycosidic nature and are widely distributed in higher plants. Despite their fairly large structural diversity, these compounds share some unique biological properties like the ability to lyse erythrocytes (Francis *et al*., 2002). A previous study reported that saponins have cytotoxic or anti-tumor activities (Podolak *et al*., 2010).

Terpenes can be chemically classified as alcohols, hydrocarbons, ketones, and epoxides with low cytotoxicity (Mendanha *et al*., 2013). A previous study reported that flavonoids have no teratogenic effects on pregnant mice or their pups (Lesser *et al*., 2015).

## CONCLUSION

The components that contribute to the anomalies are not known; phenols, tannins, saponins, and terpenes could be involved. Further studies are required to confirm this and examine these components in depth. In conclusion, Proximol should be administered with caution during pregnancy.

## ACKNOWLEDGMENTS

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**ABDELRAHMAN, H. A.; OMAR, A. R. & SALAH EL-DIN, E. Y.** Impacto de la ingesta de Proximol (*Cymbopogon proximus*) en las ratas albino embarazadas y sus fetos durante el período de gestación. *Int. J. Morphol., 35(2)*:500-505, 2017.

**RESUMEN:** Halphabarol, el principio activo de Proximol, es el más potente de los cuatro antiespasmódicos presentes en la maleza desértica nacional "*Cymbopogon proximus*" o "Halfa Bar". Halphabarol es de gran utilidad para el manejo de cólicos renales y para la expulsión de cálculos ureterales, ya que causa la dilatación del uréter por debajo del sitio de cálculo mientras se mantiene el mecanismo de propulsión activa. Se realizó una evaluación de la malformación congénita por Proximol en ratas albinas gestantes durante el período de gestación. Las ratas fueron apareadas y a las ratas gestantes se les administró oralmente, del 5 al 20º día de gestación, una dosis de Proximol (0,05 mg / kg), equivalente a la dosis humana. Al día 20 de gestación, todas las ratas fueron anestesiadas para obtener datos maternos y fetales. El grupo de tratamiento mostró algunos trastornos, que pueden resumirse como retraso del crecimiento, anomalías externas, resorción embrionaria y malformación esquelética. Concluimos que la administración oral de Proximol resultó en anomalías embrionarias y malformaciones esqueléticas.

**PALABRAS CLAVE: *Cymbopogon proximus*; Halfa Bar; Malformación.**

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