Toxic effects of hydroalcoholic extract of *Citrullus colocynthis* on pregnant mice

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Summary

Citrullus colocynthis (CCT) is used in traditional medicine to inhibit the implantation of embryos. The objective of this study was to determine the number of embryos per pregnancy and the mortality rate in pregnant mice. 115 vaginal plug-positive mice were divided into 4 groups. The animals were given 30, 60 and 120 mg/kg hydroalcoholic extract of CCT until 17th day of gestation. Control group was fed with solvent. At the day 17, the animals were sacrificed and the number of pregnant mice and embryos per pregnancy were counted. We found that while in 30 mg/kg group the mean number of embryos per pregnancy was around 10, no embryo was found in other groups. Furthermore, 3 out of 30 mice in 30 mg/kg group died, while in 60 mg/kg and 120 mg/kg groups the number of death was 7 and 14, respectively. In conclusion, CCT reduces the number of embryos and increases the mortality rate in pregnant mice in a dose-dependent manner.

Key words: Pregnancy, *Citrullus colocynthis*, Embryo, Mice

Introduction

Citrullus colocynthis (CCT) is one of the native plants of the Middle East countries which is used in traditional medicine. CCT contains active substances such as saponins, alkaloids and glycosides (Abdel-Hassan *et al*., 2000). It is used as antidiabetic, antihypertensive (Ziyyat *et al*., 1997), immunostimulant (Bendjeddou *et al*., 2003) and antioxidant (Gebhardt, 2003). There have been some reports of its side effects which can limit its usage as a traditional remedy. For instance, some reports of carcinogenic effects of CCT can be found in the literature, in spite of its antioxidant activities (Habs *et al*., 1984). There are also reports of sheep and goat death after consuming the plant (Elawad *et al*., 1984). Regarding reproductive system, CCT induces infertility in both sexes (Prakash *et al*., 1985; Chaturvedi *et al*., 2003). The histoarchitecture of the testes is shown to undergo degenerative changes of seminiferous epithelium, prevention of spermatogenesis at the secondary spermatocyte stage and cytolysis (Chaturvedi *et al*., 2003). This plant also induces antiandrogenic and reversible infertility in male albino rats (Chaturvedi *et al*., 2003). In female rats, it shows anti-implantation activity (Prakash *et al*., 1985). The objective of this study was, therefore, to investigate the number of embryos per pregnancy and mortality rate of pregnant mice after treatment with various doses of hydroalcoholic extract of CCT.

Materials and Methods

Citrullus colocynthis fruit was collected from Kavar region of Shiraz, Iran. The plant was identified by the Department of Biology as CCT and a sample was deposited at the herbarium, Shiraz University. Fresh fruit was obtained from a commercial supplier during summer and dried in shadow. CCT hydroalcoholic extract was obtained by the percolation method. In summary, 100 g of powdered dried fruit was put into percolator

and 800 ml of 50% ethanol was added to the powder over three days. The flow rate of solvent was 5 ml/kg/min. The solution was collected and the solvent was evaporated. The resulting semisolid extract was collected (yield: 4.6%) and the appropriate amount was mixed with saline to obtain various concentrations.

Female BALB/c mice weighing 25–30 g were obtained from the animal house of Shiraz Medical University. The animals were randomly mated with male partners. Observation of vaginal plug was considered as day zero of gestation. One hundred and fifteen vaginal plug-positive mice were randomly divided into three experimental and one control groups. The experimental groups were given 30, 60 and 120 mg/kg (0.1 ml) of hydroalcoholic extract of CCT by Gavage and the mice in the control group was fed with 0.1 ml of saline (solvent of CCT) for 17 days. The animals were maintained in standard condition of temperature (22–24°C), 12 hrs darkness, 12 hrs lightness periods and had free access to water and food. Animals were then sacrificed under deep anaesthesia and the fetuses were removed and counted. The morphologic characteristics of fetuses were examined under stereomicroscope. The absorptive fetuses were just consisted of fetal membrane without any fetus within it. They were compared with normal fetus morphology.

Comparison of data of all experimental groups was done by Kruskal-Wallis and Mann-Whitney U tests. P-values <0.05 were considered statistically significant.

Results

The mortality and fertility rates and also the number of fetuses in different groups are summarized in Table 1. A significant increase in the mortality rate was observed in groups treated with higher doses (60 and 120 mg/kg) of CCT in a dose-dependent manner ($P<0.05$). The fertility rate was significantly reduced as the dose increased ($P<0.05$), so that with higher doses, there were no pregnant mice. The total number of pregnant mice was significantly reduced in comparison to the control group, by exposure to the extract with concentrations of 60 and 120 mg/kg, although the mean number of fetuses per pregnancy was constant. According to the data, LD_{50} of CCT was calculated to be 100 mg/kg. This dose is close to those which induce maximal infertility effects.

Discussion

As the results of the present study showed, hydroalcoholic extract of CCT causes a decrease in fertility rate in a dose-dependent manner but it has not any effects on the number of fetuses.

Prakash *et al.* (1985) showed that ethanol and benzene extracts of CCT have 60–70% anti-implantation activity which could inhibit pregnancy. Causes of pre-implantation embryo loss or abortion are multifactorial and largely have not been described (Hansen *et al.*, 2004). However, it is probable that hormonal or structural changes at gross or molecular levels of pre-implantation embryo involve in decreasing fertility rate.

CCT contains saponins, alkaloids and glycosides component (Abdel-Hassan *et al.*, 2000). It has been reported that those plants which show contraceptive properties have saponins, alkaloids, and glycosides compounds (el Izzi *et al.*, 1990). For instance, saponin from *Tetrapleura tetraptera* inhibits the luteinizing hormone

Table 1: Effects of *Citrullus colocynthis* on mortality and fertility rate and the number of embryos in rat

	No. of cases	No. of mortality in female mice (%)	No. of pregnancy in female mice (%)	Mean number of normal fetus per pregnancy	Mean number of absorptive fetus per pregnancy
Control	30	0 (0%)	11 (37%)	10.4	1
30 mg/kg	30	3 (10%)	5 (17%)	10.2	0
60 mg/kg	30	7 (25%)*	0 (0%)*	0*	0
120 mg/kg	25	14 (56%)*	0 (0%)*	0*	1

*P<0.05 compared to the control group

(LH)-releasing hormone (LHRH)-induced LH release. This could explain the antigonadotropic properties of plants which are used as natural contraceptive (el Izzi *et al*., 1990). In addition, glycoalkaloids of some plants such as potato have toxic effects which could cause the death of embryo and result in absorbed and dead fetuses (Wang, 1993). Therefore, it is probable that induction of infertility by the components of CCT be evoked by affecting the hypothalamic-hypophyseal-gonadal axes or direct toxic effect on embryo.

On the other hand, biochemical indices of serum in pregnancy and non-pregnancy conditions can lead to the following alterations:

CCT can increase serum alkaline phosphatase activity and decrease the concentration of total protein, albumin and calcium. Alkaline phosphatase has an active role during pregnancy. This enzyme exists in the placenta as well as in the endometrium (Al- Yahya *et al*., 2000).

Alkaline phosphatase is distributed mainly in the syncytial microvillus and plasma membrane of the placenta. This enzyme is a necessary prerequisite for the full differentiation of syncytiotrophoblast (Jones and Fox, 1979). In rat uterus, alkaline phosphatase was shown to be located in apical membranes and microvillus of endometrial epithelial cells with high activity on day two of pregnancy to virtually no activity on day five. At this time, the pattern of surface fold was found to divide the uterine horn into implantation segments (Winkelmann and Spornitz, 1997). Data found in the present study showed that CCT could decrease fertility rate. As alkaline phosphatase content of endometrium decreases after the second day of gestation, any compound capable to elevate alkaline phosphatase (such as CCT), may interfere with the implantation process and placental development.

There is evidence in the literature that the level of total protein and albumin may decrease in abortion and threatened abortion (Balev *et al*., 1977). On the same basis, CCT can decrease total protein and albumin (Al-Yahya *et al*., 2000). So, it seems reasonable that CCT can induce abortion and therefore, decrease fertility rate. CCT reduced fertility rate at all doses which may be related to the changes of total protein and albumin.

Extract of CCT is shown to have a marked stimulating effect on the reticulo-endothelial system and immunostimulating activity (Bendjeddou *et al*., 2003). An effective protection is provided by the trophoblast layer which not only forms a physical barrier between the mother and fetuses but also avoids the immune attack of the mother (Diwan *et al*., 2000). An abnormal immune response of the mother against the feto-placental unit may be responsible for the occurrence of recurrent spontaneous abortions (Tedesco *et al*., 1997). Therefore, CCT may decrease fertility rate by stimulating mother's immune response.

The mother mortality rate was increased in a dose-dependent manner so that with higher concentrations it was as high as 60%, and the LD_{50} of the extract was found to be 100 mg/kg of body weight. Various components of the extract might be responsible for mother's mortality. For instance, saponin extracted from CCT induced hepato-renal damage including necrosis of liver cells and renal tubules in mice (Diwan *et al*., 2000). It has been reported that the ingestion of CCT by large animals such as sheep and goat showed signs of poisoning (Elawad *et al*., 1984). In another study, the ethanolic extract of leaves of CCT at 800 mg/kg killed 60% of the treated rats (Wasfi, 1994). It seems that the toxicity observed in this study might be due to the damage to the liver, kidney and gastrointestinal tract (Elawad *et al*., 1984).

In conclusion, CCT can decrease fertility rate in a dose-dependent manner. However, at doses of 60 and 120 mg/kg, it also shows signs of toxicity. Because of the popularity of this plant in traditional medicine for treatment of some diseases such as diabetes mellitus, people should be informed of the potential risks particularly during pregnancy.

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