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Short and Long Effects of *Citrullus colocynthis* L. on Reproductive System and Fertility in Female Spague-Dawley Rats

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Abstract: Aim of this study is to investigate the toxic effects of *Citrullus colocynthis* L. (400 mg/kg/body weight) on the reproductive system after administration to female Sprague-Dawley rats weighting 250-300 g for two time periods 4 and 12 weeks. Twenty adult female rats were divided into two groups and *Citrullus colocynthis* L. were intraperitoneally injected to experimental animals in dose of 400 mg/kg/body weight. First group containing 10 rats received treatment for 4 weeks and a second group of 10 rats received the same dose of treatment for a period of 12 weeks and compared with twenty non-exposed female rats received vehicle treatment. Female rats were allowed mating with males after 10 days prior to the last administration dose. Animals were autopsied under light anesthesia after mating and several parameters were determined including: number of pregnant rats, body and reproductive organ weight, number of implantation sites, viable fetuses and resorption sites. Assessment of pregnancies in females was measured and the significance of these results was calculated using students t and Chi-square tests. The effect of *Citrullus colocynthis* L. exposure on fertility was assessed in terms of pregnant rats number, implantation sites, viable fetuses and resorption sites. Exposure to *Citrullus colocynthis* L. for 4 weeks did not have much effect on fertility. Significant decrease in the relative ovarian weights and embryo weights in rats exposed to *Citrullus colocynthis* L. were observed. Exposure to *Citrullus colocynthis* L. for a 12 weeks resulted in a reduction in the percentage of pregnancies and in the number of implantation sites when compared with controls in both treatment periods. Rats receiving 12 weeks treatment showed a decrease in ovarian weights and a decrease in viable fetus's number. These results indicate that long-term exposure of female rats to *Citrullus colocynthis* L. causes adverse effects on the reproductive system and fertility.

Key words: *Citrullus colocynthis* L., female rats, fertility, pregnancy, reproductive organs

INTRODUCTION

Since ancient time phytotherapy has been used as folk medicine to treat various diseases including fertility regulation, a fact that has been reported in the ancient literature of indigenous systems of medicine. A number of plant species have been tested for fertility regulation beginning about 50 years ago and were subsequently fortified by national and international agencies (Kamboj and Dhawan, 1982; Purohit and Daradka, 1999; Khouri and El-Akawi, 2005).

In Jordan, *Citrullus colocynthis* L. (Cucurbitaceae), locally known as *Handal* is a well recognized plant in the traditional medicine and was used by people in rural areas as a purgative, antidiabetic and insecticide (Ageel *et al.*, 1987; Aburjai *et al.*, 2007). Mediterranean *Handal* was

also known as effective medicine and was used as traditional medicine by both the old Greeks and Romans. Powder generated from the ripped fruit pulp has been used as purgative acting directly on the gastrointestinal tract a fact demonstrated by Elawad *et al.* (1984). This plant contains a number of chemical compounds including cucurbitacins A, B, C, D and α -elaterin attributed to its purgative effect (Watt and Breyer-Brandwijk, 1962; Bakhiet, 1995; Yoshikawa *et al.*, 2007). Furthermore, components such as saponin and glycoside were also found in this plant possessing a hypoglycemic effect on rabbits (Issa *et al.*, 2000). Recently, pharmacological research performed with this plant confirmed its effectiveness in the treatment of induced diabetes mellitus in rats through a significant stimulation of insulin secretion (Namila *et al.*, 2000). Toxic effect of this plant

seeds and leaves extracts was demonstrated in sheep and found to be attributed to the administration of higher doses which could eventually lead to death (Adam *et al.*, 2000). Moreover, It was reported that oral administration of this plant aqueous extract reduces certain biochemical parameters such as AST and LDH, eliminating the toxic effect of streptozotocin-induced diabetes in rats (Al-Gaithi *et al.*, 2004; Tahraoui *et al.*, 2007).

Therefore and in the light of these facts our work was conducted to monitor the effects of *Citrullus colocynthis* L. on female rat's reproductive system with emphasis on the fertility and pregnancy outcome.

MATERIALS AND METHODS

Animals: Adult female Sprague-Dawley rats (40) weighing 250-300 g were used in this study. Rats were raised in the animal house unit/Jerash National University, Jerash, Jordan, between May and October 2005, under a controlled temperature of $21 \pm 1.0^\circ\text{C}$ and 12 h light/dark cycle. Animals were feed with regular diet (manufactured by the Faculty of Veterinary Medicine at (JUST), according to standard recipes) and water was provided *ad libitum*. Female rats were randomly divided into two treatments and two corresponding control groups of 10 rats each.

Plant and treatment: *Citrullus colocynthis* L. plants were collected from Aqaba area, mature black seeds were separated manually from the pulp of the fruits, then the pulp was dried and grinded into powder. Powder was extracted by water-ethanol mixture (70/30 V/V) for 6 h following the instructions published by Nmila *et al.* (2000). This step was repeated three times then the filtrate was pooled and concentrated under vacuum keeping a temperature less than 50°C . The concentrate was dissolved in a normal saline and used. The extract, 400 mg kg^{-1} , was administered orally to rats using animal feeding intubation's needles (Popper and Sons, New York).

Experimental design: *Citrullus colocynthis* L. plant extract was dissolved in tap water and treated rats receive this extract through an intra-gastric tube administration at a concentration of 400 mg/kg /body weight as one morning dose daily.

Rats were divided into four groups of ten rats each. These groups were treated as follows:

- Group 1: were given 400 mg/kg /body weight of the crude extract of *Citrullus colocynthis* L. dissolved in 2 mL normal saline orally for four week in a daily bases.

- Group 2: were given 400 mg kg^{-1} body weight of the crude extract of *Citrullus colocynthis* L. dissolved in 2 mL normal saline orally for 12 weeks in a daily bases.
- Group 3: were given 2 mL normal saline orally every one day for 4 weeks.
- Group 4: were given 2 mL normal saline orally every one day for 12 weeks.

Twenty four hour after of the last dose, animals were weighed and autopsied under light ether anesthesia. Blood samples were collected through cardiac puncture for serum analysis using sterile syringes.

Fertility test: Routine daily observation of rats exposed to *Citrullus colocynthis* L. for clinical signs of toxicity was done. In addition, treated rats body weights were measured weekly.

After each treatment time period, treated and control groups of rats were divided randomly into subgroups of two female rats that were caged with a sexually mature male rat for ten days to allow mating. The effect of *Citrullus colocynthis* L. ingestion on the occurrence of implantation was estimated in treated and their control counterpart's female rats after the appropriate time of mating exposure. It was estimated that at least two estrous cycles have elapsed during this exposure time (Lane-Petter and Pearson, 1971).

After the estimated mating time, treated and control counterparts female rats were weighted and sacrificed by cervical dislocation under light ether anesthesia. Autopsy was performed and the following parameters in both groups were recorded: the number of implantation sites, the number of viable fetuses and the number of resorption sites. Furthermore, uterus weights, ovary weight in addition to the embryo weights were recorded.

Statistical analysis: Data was expressed as mean \pm and standard deviation (SD). The differences between *Citrullus colocynthis* L. treated and controlled groups were analyzed using Student t-test (Dixon and Massey, 1957).

RESULTS

Exposure toxicity of *Citrullus colocynthis* L.: None of the female rats used within the 4 week exposure group (group 1) showed any clinical signs of toxicity. However, one female rat exposed for 12 week treatment period with *Citrullus colocynthis* L. (group 2) died due to respiratory problem.

Table 1a: The effect of 4 week exposure to *Citrullus colocynthis* L. on fertility of female rats

Treatments	No. of pregnant females	No. of implantation	No. of viable fetuses	Rats with resorptions	Resorptions/total No. of implantation
Control	9/10	9.33±2.39	8.77±2.72	4/10 (40%)	5/84 (5.9%)
<i>Citrullus colocynthis</i> L.	8/10	8.11±3.45	8.19±0.83	5/8 (62.5%)	11/65 (16.92%)

Results are expressed as means±SEM. *p<0.05: Significantly different from the control group (Student's t-test). †p<0.05: Significantly different from the control group (Fisher exact test)

Table 1b: The effect of 12 week exposure to *Citrullus colocynthis* L. on fertility of female rats

Treatments	No. of pregnant females	No. of implantation	No. of viable fetuses	Rats of resorption sites	No. of resorption sites/ total No. of Implantation sites
Control	9/10	9.33±2.39	8.77±2.72	4/10 (40%)	5/84 (5.9%)
<i>Citrullus colocynthis</i> L.	6/10 †	7.11±3.66*	6.76±1.85*	4/6 (80%)	13/41 (31.7%)

Results are expressed as means±SEM. *p<0.05: Significantly different from the control group (Student's t-test). †p<0.05: Significantly different from the control group (Fisher exact test)

Table 2a: The effect of 4 weeks exposure to *Citrullus colocynthis* L. on maternal body, organ and embryo weights

Treatments	Final body weight (g)	Ovary weight (g) (mg/100 g B. wt.)	Uterus weight (g) (mg/100 g B.wt.)	Embryo weight (g) (mg/100 g B. wt.)
Control	268±18.67	0.37±0.05	0.53±0.01	0.34±0.04
<i>Citrullus colocynthis</i> L.	265±9.540	0.34±0.03*	0.48±0.05	0.31±0.04†

Results are expressed as means±SEM. *p<0.05, †p<0.01: Significantly different from the control group (Student's t-test)

Table 2b: The effect of 12 weeks exposure to *Citrullus colocynthis* L. on maternal body, organ and embryo weights

Treatments	Final body weight (g)	Ovary weight (g) (mg/100 g B. wt.)	Uterus weight (g) (mg/100 g B. wt.)	Embryo weight (g) (mg/100 g B. wt.)
Control	268±18.67	0.37±0.05	0.53±0.01	0.34±0.04
<i>Citrullus colocynthis</i> L.	252±6.83	0.31±0.05*	0.45±0.06	0.29±0.11†

Results are expressed as means±SEM. *p<0.05, †p<0.01: Significantly different from the control group (Student's t-test)

The effects of *Citrullus colocynthis* L. on fertility: Short term treatment with *Citrullus colocynthis* L. extract for 4 weeks revealed a slight decrease with no significant reduction in the rate of impregnation, the number of implantation sites, as well as the number of viable fetuses when compared with controls (Table 1a). A slight but not significant elevation in the percentage rate of resorption site was observed in this group when compared with controls. Furthermore, the ratio between the resorption and the total number of implantation was observed to be in a slight elevation (Table 1a).

The effect of 12 weeks exposure to *Citrullus colocynthis* L. by female rats (group 2) on the fertility indicate that there is a significant decreases in the percentage of impregnated rats in the treatment group when compared with the control counterparts (Table 1b). Moreover, Table 1b also indicates that the long term exposure to *Citrullus colocynthis* L. for 12 weeks induces a decrease in both the number of implantation sites as well as the number of viable fetuses to a statistically significant level. It is also observed that the percentage of resorption sites in treated female rats for long term period is elevated, where the ratio between the resorption sites and the number of implantation was induced greatly (Table 1b).

The effects *Citrullus colocynthis* L. on maternal organs weight and embryo weight: Table 2a shows that ingestion of *Citrullus colocynthis* L. for 4 weeks resulted in a slight

but insignificant reduction in female rat's body as well as uterine weights. A statistical significance decrease in the relative ovarian and embryo weights in this group was observed when compared with control counterparts (Table 2a).

In contrary to this, the ingestion of *Citrullus colocynthis* L. for 12 weeks resulted in a significant reduction in both the relative ovarian weight and embryo weight when was compared to controls (Table 2b). No differences were observed in the final body weigh or in the uterine weight in rats treated for 12 weeks with *Citrullus colocynthis* L. when compared with controls, in contrary a slight reduction can be noticed (Table 2b).

DISCUSSION

The animal model in this study has been previously used by several other workers to assess the adverse effects of other extract obtained from medicinal plants on reproductive functions in rat male (Khouri and El-Akawi, 2005).

This study were conducted to investigate the exposure effect of *Citrullus colocynthis* L. on the structure, fertility and the pregnancy outcome of adult female Sprague-Dawley rats. The dose of 400 mg kg⁻¹. Body weight of *Citrullus colocynthis* L. was selected to obtain broader range of information on the effects of this plant on the reproduction parameters. Two different time period were selected namely 4 and 12 weeks.

It is worthwhile to mention that to our knowledge, no work has been published in the literature that relates the effects of *Citrullus colocynthis* L. to structure, fertility and pregnancy outcome. It has been postulated however, that administration of this plant to female rats for 30 days in different dosages induces dose-dependent decrease in the size of the offspring with no toxicological effect observed (Shapira *et al.*, 1989). This is in accordance with our results which showed that the exposure of adult female rats to *Citrullus colocynthis* L. for 4 weeks had neither toxic, nor significant effects on the rat's fertility parameters or structure of the reproductive system. However, a slight decrease in the relative ovarian weights and a significant decrease in the embryo weight in rats treated for 4 weeks were observed. On the other hand, an increase in the exposure period for 12 weeks using similar dose of this plant extract revealed a significant decrease in both the relative ovarian and embryo weights when compared to controls.

Other important findings of this study showed that this plant might promote a decreased in Sprague-Dawley female rats fertility when intra-gastric administration for long period of time was applied. This was indicated by the decrease in the reproductive organ weights observed in this group of rats. However, the weights of reproductive organs were markedly decreased as shown in Table 2a and b which might be explained by the fact that the reproductive organ weights can be closely regulated by androgen hormones (Richard *et al.*, 2000). If so, we can hypothesize that this extract may act on the hypothalamic-pituitary ovarian axis which may lead to a decrease in the main hormones influencing oogenesis and subsequent pregnancy. The decrease in the weight of reproductive organs can be explained by the possible decrease in the level of androgen hormones that could be decreased in the experimental group of rats. The unexplained decrease in the ovarian weights in treated rats needs to be clarified through both hormonal and histological analysis. In addition, the future use of advanced molecular methodologies might elucidate the pathway through which this plant acts to decrease the weight of the ovaries observed in this study. These results, therefore, suggest that any disturbance of the reproductive endocrine functions may possibly and can go hand in hand with multiple sites of androgenic toxicity acting along the hypothalamic-pituitary-ovarian-uterine axis.

Other main finding of this current study was the significant reduction in the occurrence of pregnancy in rats exposed to *Citrullus colocynthis* L. for 12 weeks. This decrease may be due to long dysfunctional period of the endocrine functions that might lead to decreased

secretion of progesterone which is needed for endometrial alteration at the time of implantation and is necessary for successful impregnation (Choudhary and Steinberger, 1975; Agrawal *et al.*, 1986).

This may go hand in hand with our results indicating the significant decrease in the number of implantation sites which could lead to the decrease in viable fetus's number. We are now conducting a research to investigate the effect of *Citrullus colocynthis* L. exposure on serum progesterone levels. In conclusion, the results of the current study suggest that ingestion of *Citrullus colocynthis* L. by adult female rats causes adverse effects on fertility and reproduction.

REFERENCES

- Aburjai, T., M. Hudaib, R. Tayyem, M. Yousef and M. Qishawi, 2007. Ethnopharmacological survey of medicinal herbs in Jordan, the Ajloun Heights region. *J. Ethnopharmacol.*, 110: 294-304.
- Adam, S.E.I., A.H. Al-Farhan and A. Al-Yahya, 2000. Effect of combined *Citrullus colocynthis* and *Rhazya stricta* use in Najdi sheep. *Am. J. Chin. Med.*, 28: 385-390.
- Ageel, A.M., J.S. Mossa, M.A. Al-Yahya, M. Tariq and M.S. Al-Said, 1987. Plants Used in Saudi Folk Medicine. King Saud University Press, Riyadh.
- Agrawal, S., S. Chauhan and R. Mathur, 1986. Antifertility effects of embelin in male rats. *Andrologia*, 18: 125.
- Al-Ghathithi, F., M.R. El-Ridi, E. Adeghate and M.H. Amiri, 2004. Biochemical effects of *Citrullus colocynthis* in normal and diabetic rats. *Mol. Cell Biochem.*, 261: 143-149.
- Bakhiet, A.O. and S.E.I. Adam, 1995. An estimation of *Citrullus colocynthis* toxicity for chicks. *Vet. Hum. Toxicol.*, 37: 356-359.
- Choudhary, A. and E. Steinberger, 1975. Effect of a reduced androgen on sex accessory organs, initiation and maintenance of spermatogenesis in the rat. *Biol. Reprod.*, 12: 609-617.
- Dixon, W. and F.J. Massey, 1957. Introduction of Statistical Analysis, McGraw Hill Book Co., Ubs, New York, pp: 228.
- Elawad, A.A., E.M. Abdel Bari, O.M. Mahmoud and S.E. Adam, 1984. The effect of *Citrullus colocynthis* on sheep. *Vet. Hum. Toxicol.*, 26: 481-485.
- Issa, A., A. Hassan, J. Ahmed, A. Barry and S.T. Mohammeda, 2000. The hypoglycaemic and antihyperglycaemic effect of *Citrullus colocynthis* fruit aqueous extract in normal and alloxan diabetic rabbits. *J. Ethnopharmacol.*, 71: 325-330.

- Kamboj, V.P. and B.N. Dhawan, 1982. Research on plants for fertility regulation in India. *J. Ethnopharmacol.*, 6: 191-226.
- Khoury, N.A. and Z. El-Akawi, 2005. Antiandrogenic activity of *Ruta graveolens* L. in male Albino rats with emphasis on sexual and aggressive behavior. *Neuro. Endocrinol. Lett.*, 26: 269-275.
- Lane-Petter, W. and A.E.G. Pearson, 1971. In: *The Laboratory Animal Principles and Practise*. London: Academic Press Inc., pp: 226.
- Nmila, R., R. Gross, H. Rchid, M. Roye, M. Manteghetti, P. Petit, M. Tijane, G. Ribes and Y. Sauvaire, 2000. Insulinotropic effect of *Citrullus colocynthis* fruits extract. *Planta Med.*, 66: 418-423.
- Purohit, A. and H.M.M. Daradka, 1999. Antiandrogenic efficacy of *Curcuma longer* (50 %EtOH extract) with special emphasis on testicular cell population dynamics. *Indian Drugs*, 36: 142-143.
- Richard, A.F., R.E. Dewar, M. Schwartz and J. Ratsiraron, 2000. Mass change, environmental variability and female fertility in wild *Propithecus verreauxi*. *J. Hum. Evol.*, 39: 381-391.
- Shapira, Z., J. Terkel, Y. Egozi, A. Nvska and J. Freidman, 1989. Abortifacient potential for the epigeal parts of *Artemisia herba-alba*. *J. Ethnopharmacol.*, 27: 319-325.
- Tahraoui, A., J. El-Hilaly, Z.H. Israili and B. Lyoussi, 2007. Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in south-eastern Morocco (Errachidia province). *J. Ethnopharmacol.*, 110: 105-117.
- Watt, J.M. and M.G. Breyer-Brandwijk, 1962. *The Medicinal and Poisonous Plants of Southern and Eastern Africa. Being an Account of Their Medicinal and Other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal*. 2nd Edn., Edinburgh E and S Livingstone Ltd.
- Yoshikawa, M., T. Morikawa, H. Kobayashi, A. Nakamura, K. Matsuhira, S. Nakamura and H. Matsuda, 2007. Bioactive saponins and glycosides. XXVII. Structures of new cucurbitane-type triterpene glycosides and antiallergic constituents from *Citrullus colocynthis*. *Chem. Pharm. Bull.*, 55: 428-434.