Effect of *Citrullus colocynthis* Medium on *in vitro* Oocytes Maturation Rate


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Abstract
The traditional IVF cycles involve ovarian stimulation protocols. Hormones treatment may increase the rate of incidence of Ovarian Hyper-stimulation syndrome (OHSS). *In vitro* maturation (IVM) of oocytes can be the alternative method. Therefore, this study aimed to investigate the influence of *Citrullus colocynthis* (*C. colocynthis*) extract on improving the in vitro maturation rate of oocytes. The result of this study showed that there was an improvement in oocytes maturation after one day treatment. Data showed a significant increase in the percentage of oocytes maturation when they treated with *C. Colocynthis* extract compared with control group \(P < 0.05\). In conclusion, this finding recommended that the IVM as an alternative, since it is simple and less stressful procedure for the patient. Add to that, the IVM procedure holds a considerable promises to assisted reproductive technologies (ART).

Keywords: *In vitro* maturation, *Citrullus colocynthis*, Oocytes, ART, Extract


Introduction
Over the past decades, IVF has witnessed dramatic advances in ART. Since the emerge of IVF in 1978, the progressive development of IVF has increased the chances for infertile couples to have a baby[1]. However, IVF has several
disadvantages which will be explained in more details later. Recently, the concept of IVM has become another option for fertility treatment. IVM is a simple procedure, initially, it is the retrieval of immature oocytes from unstimulated ovaries, followed by in vitro maturation [2].

The uses of medicinal herbs in ART have been widely investigated for their wide range of pharmaceutical activities including: antibacterial, antidiabetic, antioxidant activities and some other different activities[3-5]. Many studies have investigated the therapeutic effect of C. colocynthis extract in IVF for the ultimate goal of improving the fertility outcome[6,7]. In fact, the effect of C. colocynthis in IVM has not well addressed. Therefore, this study has highlights the impact application of C. colocynthis extract in IVM and its potential on improving the outcomes of fertility.

Materials and Methods

The Preparation of Aqueous Extract of C. colocynthis

C. colocynthis fruit was obtained from the local market and was ground into powder. The aqueous extract of C. colocynthis was prepared as described by Aggarwal et al. [8]. Briefly, 50gm of C. colocynthis powder was added to 250 ml of distilled water and run for 3 hours using the reflex device, to ensure the powder has completely dissolved. Then, the mixture was filtrated by using a sheet of gauze and the filtrated solution was collected in a clean suitable flask.

The filtrated solution was transferred into sterile Petri dishes and incubated in the incubator at 37 °C for 2 days to assure the complete drying of the filtrated solution. The working solution of 5% of C. colocynthis extract was prepared by adding 5 mg of C. colocynthis dried powder to 10 ml of DMEM Ham’s F12 Media (Sigma-Aldrich, USA). The working solution of 5% was filtered by using filters with pore size 0.45µm and 0.22µm. PH was adjusted at 7.2-7.4.

Animal Studies and Experimental Design

The animal studies were performed according to the ethical approval suggested by the University committee. The female mice with age of 8-12 weeks old and 25-30 gm of weight were obtained from the Institute of Diagnosis and Assisted Reproduction Technology at AL-Nahrain University.

The immature oocytes were collected under sterile condition using a laminar airflow hood. The female mice were sacrificed by cervical dislocation at approximately 15-18 hrs post-human chorionic gonadotropin (hCG) injection, the oviducts were isolated. The ampulla was teared up to release the cumulus- oocytes complexes (COCs).

Groups of 50 COCs were washed three times with Dulbecco's phosphate-buffered saline (DPBS; Invitrogen). Then cultured in well-dishes (Nunclon) in maturation medium with 5% C. colocynthis extract media or culture media only without 5% C. colocynthis extract (i.e. DMEM Hams F12 only) as a vehicle-treated control group. The immature oocytes were incubated in an incubator at 37 °C with 5% CO₂ and 95% humidity for one day. The number of mature oocytes was counted and the maturation percentage was calculated.

Statistical Analysis
GraphPad Prism 8.0.2 software was used for data analysis. The relationships between parameters and differences between the two groups were tested for significance using the Unpaired t-test with Welch's correction. Data are shown as Means± standard error of the mean (M±SEM). Differences of $P$ values of $<0.05$ were considered significant.

### Results and Discussion

Since the establishment of IVF technique in 1978 by Sir Robert Edwards and Patrick Steptoe, and it has become the major treatment of infertility and the most applied treatment option of assisted reproductive technology[9]. However, despite these advantages of IVF, it has several disadvantages including; the success of IVF is not guaranteed, high likelihood of multiple pregnancies, the traditional IVF cycles involves ovarian stimulation with hormones which may increase the risk of OHSS and IVF treatment can be expensive. IVM has advantages over IVF as the process of retrieval of immature oocytes doesn’t involve the stressful hormonal treatment. Thus, it could reduce the chances of OHSS incidence and also, reduce the drug cost and burden on patients[10].

Hence, this study has proposed the retrieval of immature oocytes from female mice subsequently followed by IVM, as an alternative strategy to avoid side effects of traditional IVF cycles.

The implication of medicinal herpes in ART has been widely investigated. In this study, we have investigated the beneficial effect of *C. colocynthis* extract on in vitro maturation of immature oocyte retrieved from mice. Many studies have shown that *C. colocynthis* has several pharmacological activities including; anti-inflammatory, antibacterial, antioxidant and free radical scavenging, hypoglycemic, hypolipidemic, anti-alopecia and antidiabetic[11]. Furthermore, the beneficial therapeutic effect of *C. colocynthis* on the reproductive system has been examined. Several studies have shown that fruit extract administration has a significant effect on improving sperm density[6].

Our study on mice oocyte maturation found that 5% *C. colocynthis* extract has improved the rate of maturation of oocytes. Data showed a significant effect of 5% *C. colocynthis* extract on increasing the rate of in vitro maturation oocytes when compared with vehicle-treated control group $P<0.05$ (Figure 1). The beneficial therapeutic effect of *C. colocynthis* may ascribe to it is pharmaceutical and bioactive activates mentioned earlier. Further suggested studies still needed to be conducted to investigate the mechanism of actions of *C. colocynthis* to exert its potential therapeutic effects.
Figure 1: C. Colocynthis Extract has a Potential Therapeutic Effect on % of IVM Oocytes. Data obtained in this study showed there was a significant increase in the percentage of IVM oocytes **p = 0.009.

Conclusion

In conclusion, the major aim of this study is to boost improvement in fertility outcomes. The study encourages the concept of the advantages of IVM over IVF, as the former has a better efficacy include those at risk of OHSS. Additionally, this study has emphasized the impact bioactive effect of C. colocynthis extract for ART and encourage further investigation to be conducted.

Conflicts of interest: None of the authors have any conflicts of interest relevant to this research subject.

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