

How to Cite:

Fadhil, L. A., Al-Dujaily, S. S., & Abbas, A. K. (2022). Role of activin a hormone, betacellulin (BTC) growth factor, and Heparin binding epidermal growth factor on mice embryonic development after oral administration of glycyrrhiza glabra. *International Journal of Health Sciences*, 6(S6), 7471–7479.
<https://doi.org/10.53730/ijhs.v6nS6.12094>

Role of activin a hormone, betacellulin (BTC) growth factor, and Heparin binding epidermal growth factor on mice embryonic development after oral administration of glycyrrhiza glabra

Lamyaa Abdulridha Fadhil

College of science, University of Baghdad, Baghdad, Iraq

*Corresponding author email: Lamyaa.Fadel1102@sc.uobaghdad.edu.iq

Saad Salih Al-Dujaily

High Institute for Infertility Diagnosis and ART, Al-Nahrain University, Baghdad, Iraq

Email: amal.khudair@sc.uobaghdad.edu.iq

Amal Khudair Abbas

College of Science, University of Baghdad, Baghdad, Iraq

Email: drsaadaldujaily@ieirt.nahrainuniv.edu.iq

Abstract---Glycyrrhiza glabra is a herbal plant used for treatment of many infertility diseases. Its roots, a common herbal source, are described to soothe fetuses in pregnant mice, but there isn't enough research to know if it's safe to take during pregnancy and its effects on implantation factors. This study examines the impact of the growth factors betacellulin (BTC), heparin-binding epidermal growth factor, and activin A hormone on embryonic development after oral administration of Glycyrrhiza glabra aqueous extract to mice. In line with clinical use, an aqueous extract of Glycyrrhiza glabra roots was prepared. Pregnant mice were randomly assigned to one of four groups: Two mice groups 8 and 28 weeks old treated with water as a negative control, mice aged 8 weeks old treated with aqueous extract from one of two groups for 10 days, and mice 28 years old treated with aqueous extract for 10 days. On study groups, the parameters of certain implantation factors such serum progesterone and Activin A hormones, Heparin-binding epidermal growth factor and betacellulin (BTC) growth factor were evaluated. The four groups had substantial differences in parameters. The results clearly show that mice treated with Licorice (Glycyrrhiza glabra) extraction (G group) at ages 8 and 28 weeks had significantly higher levels of progesterone hormone and

heparin binding growth factor than healthy controls, and the results revealed that mice treated with Licorice extraction (G group) of age 8 and 28 weeks had greater BTC implantation factor levels than healthy control mice.

Keywords---glycyrrhiza glabra, implantation factor, activin A, heparin binding, epidermal growth factor.

Introduction

Infertility affects one out of every six couples globally, and is considered as the inability to get pregnant following a year of unprotected sexual intercourse (WHO,2016). Female disorders contribute for around half of the causes of infertility in couples (Vander, Borghot and Wyns, 2018). Infertility in women can be caused by a range of issues, including hormone abnormalities, Endometriosis, premature ovarian failure (POF), hyperprolactinemia (excess prolactin), uterine fibroids, and pelvic inflammatory disease (PID), polycystic ovary syndrome (PCOS). are all disorders caused by hormonal imbalances (Mustafa et al., 2019). The medicinal herbs considered can enhance fertility in a number of ways, including by stimulating the hypothalamic–pituitary–gonadal axis (HPG axis) and interfering with estrogen receptors. The herbal plants can also prevent sexually transmitted bacterial, viral, and fungal infections, as well as inflammatory responses, hypersensitivity, and autoimmune diseases, by providing optimal nutritional circumstances for ovulation, implantation, uterine embryo tolerance, and fetal development (Bazm et al., 2019; Jiang et al., 2019). For many years, licorice (*Glycyrrhiza glabra*) has been used to cure a variety of ailments. Licorice has been shown to have a variety of biological effects, including significant antioxidative, antifatigue, antibacterial, antiviral, antiproliferative, and estrogenic action in various studies. As a result, some research have indicated that licorice extract might be used to treat infertility (Shamsi et al., 2020). Thus, this study aimed to evaluate the effect of *G. glabra* extraction on implantation factors that may effect on embryo developments in mice model.

Materials and Methods

Provide Plant collection: *Glycyrrhiza glabra* was taken from numerous locations in Baghdad/Iraq throughout its blossoming season. It was assigned a classification based on a reference to medicinal plant classification. The herbariums also identified and recorded a voucher specimen of the plant. of the University of Baghdad's College of Sciences. The roots of plant specimens were separated from their shoots, cleaned in tap water, and dried for seven days in the shade (Harborne, 1984).

Glycyrrhiza glabra extraction

AL-approach Saadon's was used to extract flavonoids, protein, and non-protein extracts from the *Glycyrrhiza glabra* plant (Abed AL-Saadon, 2005).

Animals Experimentation

Forty fertilized White female mice weight 19 to 23 grams were purchased from the University of Baghdad's, College of Veterinary Medicine. The animals had access to food and water and were housed in standard cages (a typical laboratory pellet diet). The animal housing had a 12-hour light/dark cycle and had a temperature range of 24 to 29 °C. The investigation's protocol was approved by the institutional animal ethics committee (IAEC).

Design Experimentation

The white female mice were divided randomly into (4) groups giving the following treatment for mice age 8 weeks old and 28 weeks old:

- **Group 1:** The control group was fed with 0.2 ml distilled water only, for mice age (8) weeks old. .
- **Group 2:** Glycyrrhiza glabra (G) group: fed only with (25 mg/ daily) of licorice extract dissolved in 0.2 ml of distilled water, for mice age (8)weeks old.
- **Group3:** The control group was fed with 0.2 ml distilled water only, for mice age (28) weeks old. .
- **Group 4:** Licorice Glycyrrhiza glabra (G) group: fed only with (25 mg/ daily) of licorice extract dissolved in 0.2 ml of distilled water, for (28) weeks old.

Hormonal and implantation factors study

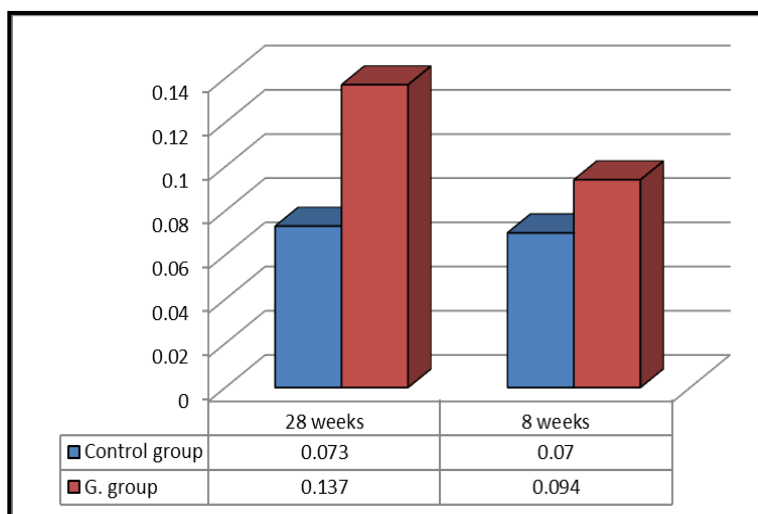
The levels of serum progesterone and Activin A were measured by Enzyme Linked Immuno Sorbant Assay (ELISA) kits provided by Abcam Company. BTC was evaluated through using ELISA Kit provided by CUSABIO USA. While Heparin binding epidermal growth factor measured through using ELISA Kit obtains by BIOVISION Company, USA. We used the manufacturer's directions about every procedure.

Analytical statistics

For the statistical study, SPSS v.28.0 statistical analysis software was employed (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) and Dunnett's multiple comparison test were used to undertake statistical studies of biochemical parameters. The Chi-square test and the Kruskal-Wallis test were used to analyze specific biochemical testing. If the p value was less than 0.05, the results were considered statistically significant.

Results

Figure 1 shows the results of progesterone hormone levels among study groups. The results revealed a significant higher mean of progesterone hormone in mice group treated with Glycyrrhiza glabra extraction (G group) of age 8 and 28 weeks (0.094 and 0.137 ng/ml , respectively) compared to healthy control group of corresponding ages (0.07 and 0.073 ng/ml , respectively) as shown in figure 1.

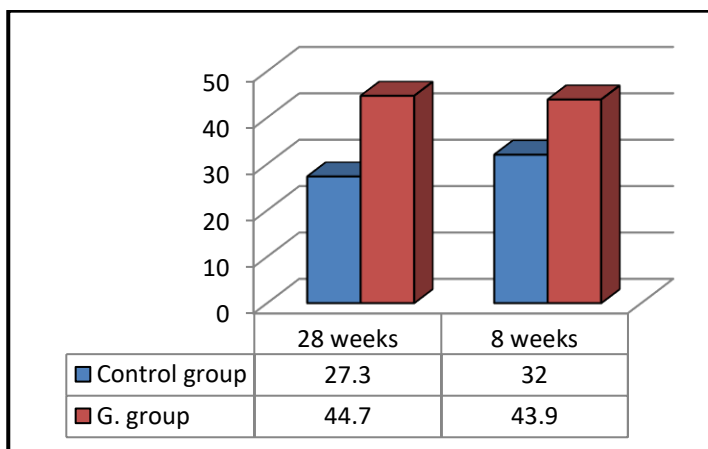


Similar letter mean no difference significant at 0.05 (t-Test)

Different letter mean difference significant at 0.05 (t-Test)

Figure 1. Comparison the Levels of progesterone hormone between mice age 8 and 28 weeks old

The results of heparin binding growth factor levels among 8 and 28 mice age groups has been shown in figure 2. The results revealed significantly higher mean of heparin implantation factor in mice group treated with Licorice extraction (G group) of age 8 and 28 weeks (43.9 and 44.7 ng\ml ,respectively) than healthy control group of age 8 and 28 weeks (32 and 27.3 ng/ml) respectively (Figure 2).



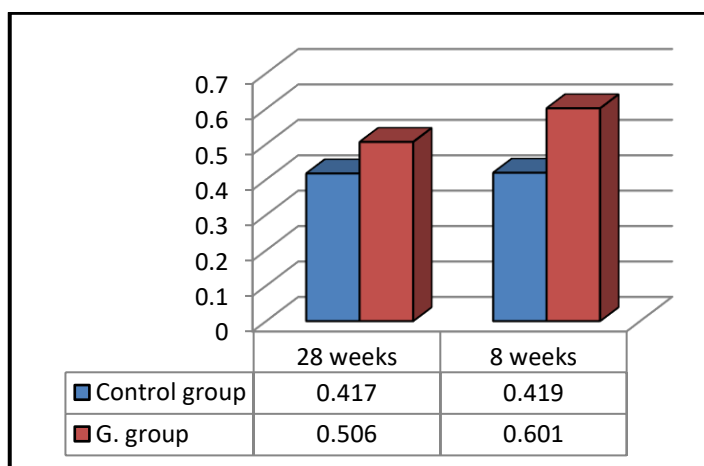
Similar letter mean no difference significant at 0.05 (t-Test)

Different letter mean difference significant at 0.05 (t-Test)

Figure 2. Comparison the Levels of heparin binding growth factor between mice age 8 and 28 weeks old

In figure 3,the results shown significantly higher increase of BTC implantation factor in mice group treated with Licorice extraction (G group) in mice age 8 and

28 weeks (0.601 and 0.506 ng/ml, respectively) compared to healthy non treated group of age 8 and 28 weeks (0.419 and 0.417 ng/ml, respectively) as illustrated in figure 3.

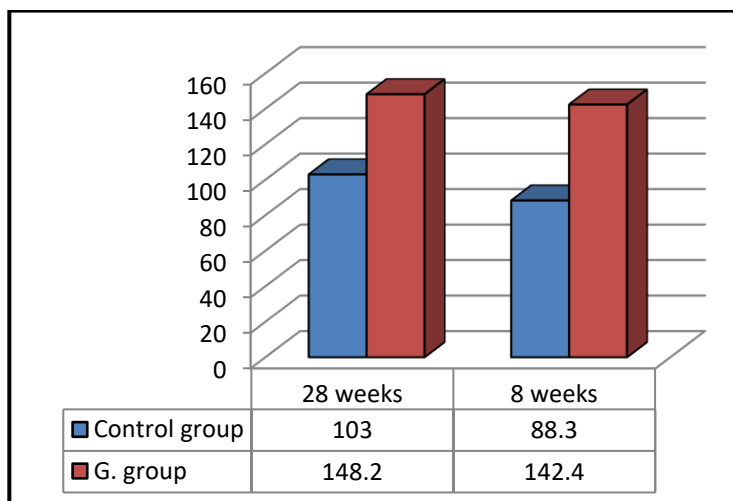


Similar letter mean no difference significant at 0.05 (t-Test)

Different letter mean difference significant at 0.05 (t-Test)

Figure 3. Comparison the levels of Betacellulin (BTC) implantation factor between mice age 8 and 28 weeks old

The results showed significantly higher level of Activin A hormone in mice group treated with G- glabra extraction (G group) of age 8 and 28 weeks (142.4 and 148.2 ng/ml, respectively) than that in healthy control group of the same ages (103, 88.3 ng/ml, respectively) as shown in figure- 4.



Similar letter mean no difference significant at 0.05 (t-Test)

Different letter mean difference significant at 0.05 (t-Test)

Figure 4. Comparison the levels of Betacellulin (BTC) implantation factor between mice age 8 and 28 weeks old

Table 1 shows the results of embryonic grading score in mice age (8) and (28) weeks old treated with Glycyrrhiza glabra extraction. The data found a significant ($p<0.05$) improvement average number of embryos grade (1) (9 ± 0.57) of Gg group compared with control group with age 8 weeks, followed by grade (2) (10 ± 0.42). While the higher mean was grade (4) (16 ± 0.51), as shown in table 1. With mice age (28) weeks, the results shows a significant ($p<0.05$) higher mean number of grade (4) (15 ± 0.72) of Gg group compared with control group (16 ± 0.72), followed by grade (3) (13 ± 0.60). While the lower mean number was grade (1) (10 ± 0.45) (Table 1).

Table 1
Embryonic grading score (blastula) after mating of female mice age 8 and 28 weeks old

Mice groups						
Age	8 weeks old			28 weeks old		
Blastula grades	Control	Gg	LSD value	Control	Gg	LSD value
Grade 1	9 ± 0.42 a	9 ± 0.57 a	1.263 NS	8 ± 0.35 b	10 ± 0.45 a	1.631 *
Grade 2	9 ± 0.42 a	10 ± 0.42 a	1.502 *	10 ± 0.51 ab	12 ± 0.63 a	2.267 *
Grade 3	15 ± 0.63 a	13 ± 0.65 ab	2.083 *	13 ± 0.59 a	13 ± 0.60 a	1.381 NS
Grade 4	17 ± 0.75 a	16 ± 0.51 a	1.944 *	16 ± 0.72 a	15 ± 0.72 ab	2.097 *

*Means having with the different letters in same row differed significantly ($P \leq 0.05$).

Discussion

The current study found that consuming licorice extract orally can raise levels of progesterone and BTC levels and significantly accelerate mouse embryo development, oocyte maturation, and fertilization rates. This study is harmony with other studies that documented licorice extraction effects on implantation factors and sex hormones such as progesterone, estrogens, and testosterone (AL-Dujaily et al., 2009; Mohsin and Al-Dujaily, 2019). On other side, Armanini et al., (2004), documented that licorice treatment did not significantly alter progesterone or LH levels. Progesterone plays an important factor in fertilization by signaling human sperm as they travel through the vaginal system before to fertilization. However, the receptor that is responsible for this pathway has yet to be discovered (Saleem et al., 2014).

It has been reported that licorice root contains the active ingredients formononetin and isoliquiritigenin, aid in IVF (Tung et al., 2015). Despite the fact that Tung and colleagues the two phytoestrogens found here may aid with fertilization because there is a tenuous link between estrogen and conception. (Tung et al., 2015). Some of the 500 components in licorice extract are thought to interact synergistically to enhance fertilization (Tung et al., 2015). It was observed

in a mouse modal research that two different doses of licorice as an antioxidant had a variety of positive effects on sex hormones and enhanced ovarian morphology (Al-Dujaily and Ali, 2015). Also this study shows that licorice extract causes significantly increase of activin A and heparin epidermal growth factor levels in mice model with age 8 and 28 weeks compared to control group. Researchers employed the HB-EGF to improve sperm fertility and embryo development. It is accepted as a standard test for investigations on assisted reproduction to employ mouse in vitro fertilization. (Wen et al., 2018). In most tissues, including those of the female reproductive system, heparin-binding epidermal growth factor exhibits pleiotropic biological roles. It is hypothesized to have a role in endometrial receptivity and maturation, as well as facilitating embryo growth and mediating implantation (Chobotova et al., 2005).

The improvement of Activin A level in current study was positively correlated with embryonic development. It has been noticed that increase activin level interfere with embryonic stem cells pluripotent and promote differentiation into specific cell lineages (Li et al., 2010). Glycyrrhiza glabra treatment to mice for days that increased the number of implantation sites might indicate modest doses of licorice have an influence on embryo development (da Rocha et al., 2018). Moreover, the current study found that oral administration of Licorice alone, the embryonic grading score (blastula) and embryo grading were improved in mice aged 8 and 28 weeks compared to the control group. This finding corroborated prior theories about increased ova maturation, normal fusogenic process, and early embryonic development. Licorice extracts may also boost the implantation rate and normal embryonic growth, resulting in an increase in the frequency of live births. It's possible that this is related to the progesterone enhancement effect, as mean corpus luteum counts have increased greatly in Gg oral administration groups, which is critical for embryo survival and pregnancy maintenance Mohsin et al. (2019). Thus, this could demonstrate the potential to be used for aged mammals, such as humans, to improve ovarian capacity and other reproductive fertility potential status while minimizing the cost and side effects of using fertility drugs, which is compatible with other research conducted by Mohsin et al. (2019) and Wahab et al. (2021). Licorice may serve as an antioxidant or immunomodulator, influencing fertility and fetal development by stimulating the synthesis of insulin-like growth factor (IGF-1) in target tissues. IGF-1 is a key regulator of fetal organ development and growth (Hellström et al., 2018).

Conclusion

In conclusion, giving a high dose of Glycyrrhiza glabra aqueous extract to adult female mice decreases implantation loss and increase litter size. The results of this research back up the traditional usage of licorice (Glycyrrhiza glabra) extract to boost female fertility.

References

Abed AL-Saadon, M. 'Isolation of Some Compounds from Celery (Apium graveolens) Seeds and Studying their Effects in Mice Exposed to Oxidative

- Stress', Ph.D. Thesis, College of Education. University of Mosul, Mosul, Iraq. 2005.
- Akbari Bazm, M, Khazaei, M, Khazaei, F, Naseri L. *Nasturtium Officinale* L. hydroalcoholic extract improved oxymetholone-induced oxidative injury in mouse testis and sperm parameters. *Andrologia*. 2019; 51(7), e13294.
- Al-Dujaily SS, Ali SMM. DNA normality after in vitro preparation with pentoxifylline and glycyrrhiza glabra of cryopreserved human sperms. *World J Pharm Res*. 2015; 4(4):1815-1828.
- AL-Dujaily SS. and Al-Saadi AS. Effect of Glycyrrhiza glabra extract on IVF outcome in mice: Experimental model for mammals. *Journal of Biotechnology Research Center* 2009; 3(2): 80-9.
- Armanini D, Mattarello MJ, Fiore C, Bonanni G, Scaroni C, Sartorato P, Palermo M. Licorice reduces serum testosterone in healthy women. 2004; 69(11-12), 0–766.
- Chobotova K, Karpovich N, Carver J, Manek S, Gullick WJ, Barlow DH, Mardon HJ. Heparin-binding epidermal growth factor and its receptors mediate decidualization and potentiate survival of human endometrial stromal cells. *J Clin Endocrinol Metab*. 2005; 90(2):913-9.
- da Rocha CF, Lima S, Carvalho H O, Pinto R C, Ferreira I M, Castro A N, Lima C S, Carvalho J C T. Action of the hydroethanolic extract of the flowers of *Acmella oleracea* (L.) RK Jansen on the reproductive performance of Wistar female rats: a popular female aphrodisiac from the Amazon. *J. Ethnopharmacol*. 2018; 214:301–308.
- Gandamay, I. B. M., Antari, N. W. S., & Strisanti, I. A. S. (2022). The level of community compliance in implementing health protocols to prevent the spread of COVID-19. *International Journal of Health & Medical Sciences*, 5(2), 177-182. <https://doi.org/10.21744/ijhms.v5n2.1897>
- Harborne JB. *Phytochemical methods: A guide to modern techniques of plant analysis*. 2nd ed. London: Chapman and Hall ltd;1984. 288p.
- Hellström A, Ley D, Hansen-Pupp I, Hallberg B, Ramenghi LA, Löfqvist C, Smith LE, Hård AL. Role of insulinlike growth factor 1 in fetal development and in the early postnatal life of premature infants. *Am J Perinatol*. 2016;33(11):1067.
- Jiang, D., Li, L., & Zeng, B. Y. (2019). Treatment of Chinese herbal medicine for female infertility. *International Review of Neurobiology*, 1(135), 233– 247.
- Li S, Shimono C, Norioka N, Nakano I, Okubo T, Yagi Y, Hayashi M, Sato Y, Fujisaki H, Hattori S, Sugiura N, Kimata K, Sekiguchi K. Activin A binds to perlecan through its pro-region that has heparin/heparan sulfate binding activity. *J Biol Chem*. 2010; 285(47):36645-55.
- Mohsin AR, Al-Dujaily SS, Haba MK. In Vitro cytotoxicity evaluation of novel combined plant crude aqueous extract of licorice and Maca, *Ann Trop & Public Health* 2019; 22(8): S224.
- Mustafa M, Sharifa A M, Hadi J, Ilzam E, Aliya S. Male and female infertility: Causes, and management. *IOSR Journal of Dental and Medical Sciences* 2019; 18, 27– 32.
- Saleem MM, Sulaiman GM, Mohammad AA, Mohammad AA. Biological Effects of Stick Cherry, Soybean Seed and Licorice Root Extracts on Concentration of Serum Hormone Levels in Male Mice. *Al-Nahrain Journal of Science* 2014;17(4):18-26.
- Shamsi M, Nejati V, Najafi G, Pour SK. Protective effects of licorice extract on ovarian morphology, oocyte maturation, and embryo development in PCOS-

- induced mice: An experimental study. *Int J Reprod Biomed*. 2020;18(10):865-876.
- Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. *International Journal of Health Sciences*, 5(3), x-xv. <https://doi.org/10.53730/ijhs.v5n3.2938>
- Tung NH, Shoyama Y, Wada M, Tanaka H. Two activators of in vitro fertilization in mice from licorice. *Biochem Biophys Res Commun*. 2015; 467: 447–450.
- Tung NH, Tanaka H, Tsujimura A, Miyagawa Y, Wada M, Fujii S, et al. In vitro fertilization with mouse sperm activated by components of licorice root extract. *Nat Prod Chem Res*. 2016; 4: 1–4.
- Vander Borgh M, Wyns C. Fertility and infertility: Definition and epidemiology. *Clinical Biochemistry* 2018; 62, 2– 10.
- Wahab S, Annadurai S, Abullais SS, Das G, Ahmad W, Ahmad M F, Kandasamy G, Vasudevan R, Ali MS, and Amir M. *Glycyrrhiza glabra* (Licorice): A Comprehensive Review on Its Phytochemistry, Biological Activities, Clinical Evidence and Toxicology. *Plants* (Basel, Switzerland) 2021; 10(12), 2751.
- Wen Y , Yan Y , Xiaoyan W , Fang W , Qian S , Yuan G, Huanhuan Z , Wen F , Ning M. Effect and mechanism of low molecular weight heparin calcium on mouse embryo implantation. *Chinese Journal Of Comparative Medicine* 2018; 28(12): 61-67
- World Health Organization. General guidelines for methodologies on research and evaluation of traditional medicine. World Health Organization; 2000.