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ABORTIVE EFFECT OF HYDROALCOHOLIC EXTRACT OF *Astragalus Fasciculifolius* GUM IN MICE

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There is no conflict of interest

ABSTRACT

Objectives: This study aimed to explore the abortive effect of *Astragalus fasciculifolius* (*AF*) gum resin in mice model.

Background: *AF* has been demonstrated a wide range of potential therapeutic application in traditional medicine, however; some sources have been noted its consumption during pregnancy leads to abortion.

Methods: In this experimental study, adult virgin Balb/c mice were designated to three categories: pre-fertilization, pre-implantation (1-4 day of pregnancy), and implantation periods (5-7 day pregnancy). In each category, the mice were randomly assigned to four groups (n=8 group). One control and three experimental groups which to receive a daily

intraperitoneal injection of distilled water and *AF* gum resin (50, 100 and 200 mg/kg) respectively. The animals were dissected on day 11-13 and the numbers of implantation sites (IS) were determined. The Data were analyzed by one-way ANOVA, and Kruskal-Wallis tests.

Results: In per-fertilization period, the average number of IS in control, 50, 100 and 150 mg/kg dosage groups, were 11±1.1, 9.8±0.75, 7.7±3.8 and 6.2±3.4 (p= 0.024), and in the pre-implantation period were 10±1.4, 6.7 ±2.7, 3.5±2.2 and 3.8±2.7 (p=0.002); and during implantation were 9.5±1.7, 6.5±4.1, 4.2±2 and 2.5±1.7 (p=0.007), respectively.

Conclusion: *AF* showed abortive effects in higher doses; particularly in preimplantation and implantation.

Keywords: *Astragalus fasciculifolius*, Preimplantation, Implantation, Abortion

INTRODUCTION

Traditional herbal medicines have been commonly used over the years to treat illness in different countries such as Iran, and traditional medicines are particularly getting significant attention in global health practices in recent years. A recent study has shown that the use of herbal medicinal products has expanded rapidly among many women for treatment of common health issues such as anxiety, dysmenorrheal, menopausal symptoms, menstrual disorders, mood disorders and prevention of osteoporosis. Despite its enormous therapeutic potential, however, inappropriate use of herbal medicinal products could have adverse effects (1). In Contrast to the common belief that herbal remedies have no side effects, researches has revealed that consumption of these substances, particularly during pregnancy or breastfeeding period, might cause congenital malformation.

Astragalus fasciculifolius (*AF*) also known as Anzerot, Persian Kahel, Kermanian Kahel, or Konjedeh (2) belong to *Astragalus* genus and Fabaceae family (3). This genus has more than 900 species in Iran that some of them are annual plants while others are perennial plants. The *A. fasciculifolius* (*AF*) has been widely administered as herbal medicine for treatment of scorpion stings, stomach ulcers, chronic bronchitis, cough, hypertension, and diabetes (4, 5). The

most effective type of *A. fasciculifolius* gum (Anzerot) exhibits a yellowish color and is similar to frankincense in apperance. It has regenerative properties without causing any irritation, facilitates wound healing (6).

Bensky et al has shown that *AF* resin can help to reduce the symptoms associated with spleen disease, fatigue, edema, loss of appetite and dyspnea in elderly (7). These herbs treat numbness of limbs, arthritis, and pain (8). Shirkani et al. has reported that anti bacterial activity of the *A.F* extracts against *Escherichia coli* and *Micrococcus luteus* (9). *Astragalus* genus has been considered as a traditional herb may with many indications such as boosting the immune system (10), decreasing risk of cancer (11), diabetes, and protecting against influenza (12). The *Astagalus* contains three valuable component including polyphenols, polysaccharides, and saponins which have such a positive impact on human health (13-16).

Abortion can be induced by environmental and genetic factors, endocrine diseases, malnutrition, coagulation disorders, generic drugs and herbal remedies. (17), the later can be exemplified by the effect of *Falcaria vulgaris* hydro-alcoholic extracts on reduction of implantation sites in laboratory mice (18). Also, Jafarzadeh et al. showed that *Hypericum* extract (150 mg/kg body weight) has abortificient effects in mice (19). Methanol extract of the root of *henna* have an effect abortificient in mice, rats and guinea pigs, which is attributed to apigenin, the highest combination of it alcoholic extract (20). A study was reported abortive effect of *Physalis alkikenji* in mice (21). Although, *A. fasciculifolius* gum use in traditional medicine has a long history, but there is no study on the clinical aspects of *A. fasciculifolius* gum consumption during pregnancy, and its side effects. The aim of this study was to evaluate the effect of ethanol extracts of *A. fasciculifolius* gum abortificient in Swiss mice.

MATERIALS AND METHODS

Plant

A. fasciculifolius gum was identified by a botanist at the Razi University in Kermanshah, and was extracted by using percolation method (22). The About 20 grams of *AF* powder was soaked in 400 ml

of 70% ethanol, the suspension was placed on a shaker for 15 minutes to facilitate extraction of active components and stored in the dark. After 48 hours, the extract was filtered and was air-dried.

Animals

Virgin Balb/c strain mice (25-30 gr) were used in a controlled temperature 22±2° C, and relative humidity 50-60% in a 12 hours light-dark cycle with freely access to standard food and water. The abortive effect of *A. fasciculifolius* gum extract was assessed to three categories: pre-conception, pre-implanation (1-4 day of pregnancy), and implantation period (5-7 day pregnancy). In each category, the mice were randomly assigned to four groups to receive a daily (at 10 am) interaperitoneal injection of distilled water and *A. Fasciculifolius gum resin* at doses of 50, 100 and 150 mg/kg for control group and three experimental groups, respectively. Before sunset, the mice with a ratio of 3: 1 (three females and one male) were placed in a cage for mating. On the next morning, after detection vaginal plug, first day of pregnancy was considered (21).

In pre-fertilization category: A control group received distilled water and three experimental groups received single dose of 50, 100 and 200 mg/kg extract interaperitonealy, and then female mated with male. In pre-implantation period; mated female mice were received distilled water in the control group, and three experimental groups received one of three doses of the extract on days 1-5 of pregnancy. In implantation category; animal in 5-7 days of pregnancy received distilled water in control group (extract solvent) and one of extract doses.

All animals were killed by cervical dislocation on day 11 to day 13 of pregnancy, and the numbers of implantation sites (bead-like structures) were counted using binocular loop (stereomicroscope).

Statistical analysis

The data was performed using the SPSS software package (version 16). One-way analysis of variance (ANOVA) was used in case the data follow a normal distribution; otherwise, a Kruskal-Wallis method was used. P-values less than 0.05 were considered significant.

RESULTS

The objective of this study was to explore the abortificient effect of hydro-alcoholic extract of *A. fasciculifolius* gum resin in mice model. In per-fertilization (pre-conception) period, the average number of implantation sites in control group and 50, 100 and 200 mg/kg dosage groups, were 11±1.1, 9.8± 0.75, 7.7±2.8 and 6.2±2.4, respectively. Reducing the number of implantation site was statistically considerable between groups (p= 0.024) (Figure 1). In the pre-implantation period also the average number of implantation site were decreased in control group (10±1.4) and three dosage groups including 50 mg/kg (6.7 ±2.37), 100 mg/kg (3.5±2.2), and 200 mg/kg (3.8±2.7). The number of implantation site was found statistically significant in 100 mg/kg (p= 0.005) and 200 mg/kg (p=0.007) groups (Figure 2). Finally, in implantation period the lower number of implantation site was shown in control group and 50, 100, and 200 mg/kg dosage groups which were 9.5± 1.7, 6.5 ± 3.1, 4.2 ± 2, and 2.5±1.7, respectively. The average number of implantation sites between groups was significant (p=0.013). Thus, there was statistically significant correlation of lower number of implantation sites and higher dosage of *A. Fasciculifolius* gum resin, consistent with dose dependent abortive effect of this extract.

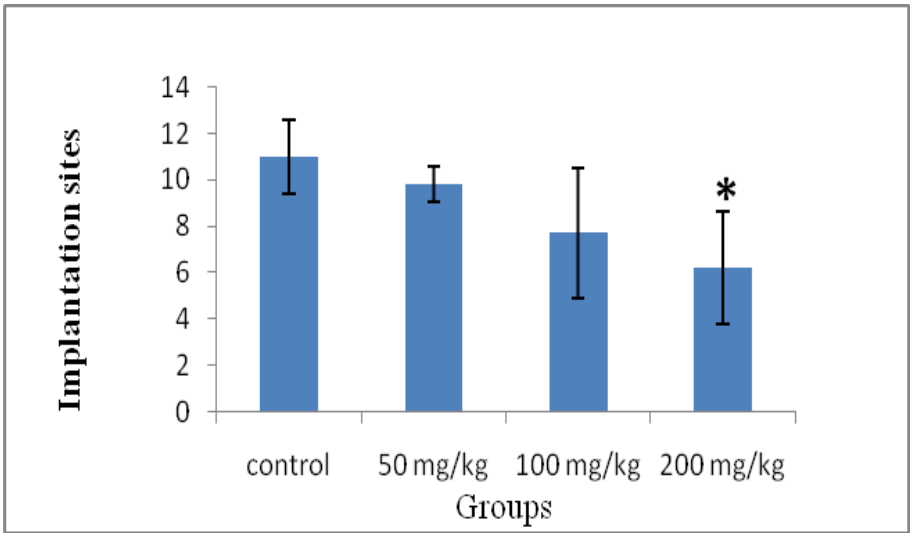

Figure 1: Mean of implantation site of pre-fertilization periods in control and experimental groups; * P=0.024

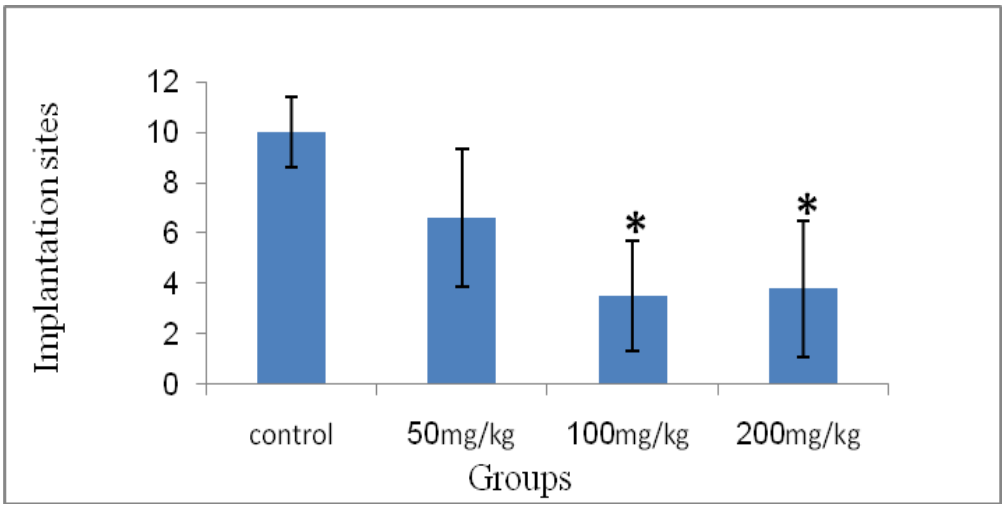

Figure 2: Mean of implantation site of pre-implantation periods in control and experimental groups. * P=0.005

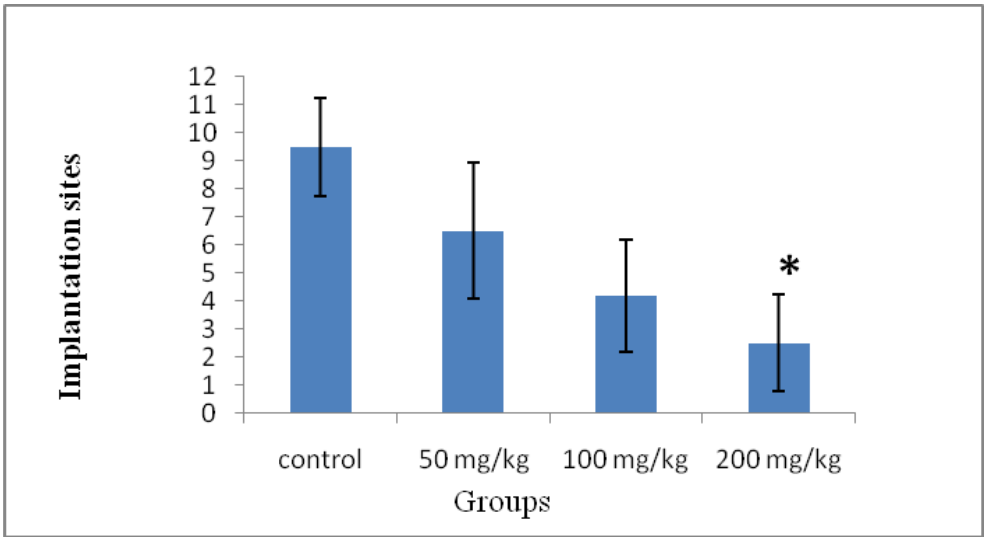

Figure 3: Mean of implantation site Of implantation periods in control and experimental groups. * P=0.013

DISCUSSION

In our study we found that hydroalcoholic extract of *A. fasciculifolius* gum decreased the number of implantation sites significantly before fertilization, pre- and during implantation in a dose-dependent manner. The extract was found to be most potent during implantation between days 5 and 7. The dose of 200 mg/kg was found to be more effective in pre-conception as well as implantation period. However, both 100 and 200 mg/kg doses prominently decreased the number

of implantation sites during pre – implantation phase.

It is observed that abortion usually occurs due to excitation and contraction of the uterus; relaxation of uterus muscles, hormonal disorders or endometrial preparation (19, 23). Though the exact mechanism by which the plant extract causes abortion is not studied, we reckon that it might trigger single or multiple factors known for its induction.

Primarily female infertility occurs because of failure to ovulate, low quality ovum, obstruction in uterine tubes and/or lack of embryo attachment to the uterine wall (24). This leads to disturbance in the structure of the endometrium. In the present study, we observed that 200 mg/kg of *A. fasciculifolius* gum extract decreased implantation sites when administrated before conception. This may be either due to the reduced ovum number or its inferior quality during its release from the ovary. We suggest that decrease in the number of implantation sites in the pre- implantation periods might be due to changes in the biochemical environment of fertilization or failure of the embryo to attach to the uterine wall. Astragalus genus plants are enriched with saponins, polysaccarides, alkaloids, β systerol and flavonoids (25). However, phytochemical analysis to detect the compounds present in *A. fasciculifolius* has not been performed. Bianco et al. reported that some alkaloids that cause aromatase (key enzyme in the synthesis of steroids) inhibition affect steroid production thus hampering the performance of the reproductive system (24).

Other studies have also shown that certain flavonoids play an important role in the regulation of the hypothalamic-pituitary-adrenal axis. These data suggest that decrease in progesterone level, inability to retain the fetus and eventually abortion related to flavonoids in *A. fasciculifolius* can be attributed to interference in hypothalamic -pituitary-ovary axis (27, 28).

Apigenin is one of flavonoids found in plants of Astragalus genus (29). Likewise, aborticifent effect of methanolic henna root extract in mice, rats and pigs is considered to be the result of apigenin in its hydroalcoholic extracts. Apigenin has slow metabolism, so as excretion and absorption phases. Therefore, possibly

accumulate in the body (30). Other potential compounds in the plant *A. fasciculifolius* as a member of Astragalus family is Apigenin which may account for abortive effect of this herb. *A. fasciculifolius* gum extracts showed abortive effect through decrease the number of implantation sites in dose-dependent manner. This plant will be used with caution during pregnancy.

Learning Points

Despite the beneficial effect of *A. fasciculifolius*, causion should be considered during its use in pregnancy.

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