Contraceptive Effects of *Onosma armeniacum* on Embryo Implantation in Rats

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ABSTRACT

O. armeniacum has traditionally been used to heal wounds, burns, dyspnea, hoarseness, hemorrhoids, abdominal aches, stomach ulcers, and gynecological problems. In this study, the embryotoxic and contraceptive effect of *Onosma armeniacum* root extract (A-1) was investigated in female rats.

Group 1: 100 mg/kg A-1 for seven days beginning at the previous day of the mating period. Group 2: 100 mg/kg A-1 for seven days beginning at first day of the mating period. Group 3: 100 mg/kg A-1 for seven days beginning 48 hours after the mating period Group 4: 100 mg/kg A-1 for seven days beginning 120 hours after the mating period rats. Group 5: Sunflower oil + 3 days mating period (Control group). Also histopathological examinations were performed on ovary and uterus of A-1 given rats.

Histopathology of ovary and uterus in the rats were not affected by A-1 supplementation.

A-1 did not induce toxic effect on embryo although it induced contraceptive effects on the embryo implantation in the rats. Shikonin content of the A-1 may be responsible on the contraceptive effect in the rats.

Key words: Onosma armeniacum; embryotoxicity; contraception; shikonin; rat.

ABBREVIATIONS

O. : Onosma
A-1 : Code of the *Onosma armeniacum* root extract
HPLC : High Performance Liquid Chromatography
DAD : Diode-Array Detector
UV : Ultraviole
PNL : Polymorphonuclear Leukocytes

INTRODUCTION

The genus *Onosma (Boraginaceae)* is known to include more than 150 species (El-Shazly et al. 2003). Among these, *Onosma armeniacum* Klokov grows in Turkey, especially in East Anatolia Region (Riedl 1978). The roots of some Onosma species contain naphthoquinones (Khajuria and Jain 1993, Ozgen et al. 2004a). Isolation studies has shown that O. argentatum, another Onosma species, contains deoxyshikonin, acetyl shikonin, 3-hydroxyisovaleryl shikonin, and 5,8-0-dimetyl acetylshikonin (Ozgen et al. 2004a). Alkannin, shikonin and alkannin/shikonin derivatives have been shown to exert antibacterial, antifungal, antiameobic, anti-inflammatory, anti-cancer and wound-healing effects (Papageorgiou et al. 1999). *Onosma armeniacum* has traditionally been used as a folk medicine in Turkey to treat has traditionally been used to heal wounds, burns, dyspnea, hoarseness, hemorrhoids, abdominal aches, stomach ulcers, and gynecological problems (Ozgen et al. 2004b). An extract, used orally, is prepared from the roots of *Onosma armeniacum* by villagers who heat the roots with butter and then filter (Ozgen et al. 2004b). In an experimental study of Cadirci et al., *Onosma armeniacum* has shown to possess antiulcer and antioxidative effects; in this study, it has been also shown that up to 2500 mg/kg dose, *O. armeniacum* does not cause mortality in any rat (Cadirci et al. 2007). Several drugs have embryotoxic and teratogenic effects which preclude their use in the first trimester of the pregnancy (Kayaalp 2002) and some plant extracts have also been found to have the same effects (Yang 1989, Kennelly et al. 1999). Thus, it is essential that any herbal medicine used as a folk remedy be examined with regard to embryotoxic and other toxic effects.

Our literature search did not reveal any scientific studies on embryotoxic effects of *O. armeniacum*, which has been widely reported to be used as a folk remedy against wounds, burns, lip and hand cracks, stomach pain and ulcers, and gynecological problems worldwide. The objective of the present study was to determine whether *Onosma armeniacum* root extract referred to as A-1 possess embryotoxic effects or not.

MATERIALS AND METHODS

Animals

A total of 70 albino wistar rats (20 male, 50 female) obtained from Medical Experimental Research Centre, Atatürk University and weighing between 230 and 240 grams were used for this study. The animals were fed under normal conditions (22 °C) in separate

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Received : November, 1, 2009
Accepted : November, 12, 2009

groups. Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals and approved by the local animal care committee of Atatürk University.

Experimental Groups

A total of 50 young, sexually inactive, female rats weighing between 70 and 87 g were fed in the same cage for 75 days until they reached a weight of 230-240 g. In this period rats completed maturation and became suitable for pregnancy. Next, the female rats were assigned into 5 groups in equal numbers, and 4 male rats were also placed into each cage.

Plant material

The roots of *Onosma armeniacum* were collected from Çiğdemli village (Ilica District, Erzurum province, Turkey) in June 2005. A voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy, Ankara University, Ankara (AEF 23796). It was authenticated by Dr. Ufuk Ozgen, Faculty of Pharmacy, Pharmacognosy Department, Atatürk University.

Extraction method

The roots (100 g) of *Onosma armeniacum* were powdered and extracted with 3×500 ml n-hexane–dichloromethane mixture (1:1) under reflux for 3 h for each extraction. The combined extracts were evaporated under reduced pressure to give concentrated extract A-1.

Instrumental analysis

Deoxyshikonin, acetyl shikonin, and 3-hydroxy-isovaleryl shikonin were isolated from extract A-1 using n-hexan:EtOAc mixture with gradient elution on a silica gel column. Final purification was performed by preparative thin layer chromatograhpy.

To determine the relative contents of the extract HPLC analysis was performed with the presence of standard samples: 3-Hydroxy-isovaleryl shikonin, acetyl shikonin and deoxyshikonin (Figure 1a, 1b, 1c).

HPLC analysis was performed on a Thermoquest HPLC system, equipped with a DAD detector (Thermo UV 6000 diode-array). For all analysis, a RP-C18 column (250x4.6 mm, 5 μm particle size, ACE®) was used. The mobile phase consisted of acetonitril-methanol-water (2% acetic acid) (60:20:20, v/v), which were applied in the isocratic elution. The analysis temperature was kept constant at ambient temperature, flow rate and sample volume were set to 1 ml/min and 20 μl, respectively. All analysis were monitored at 525 nm. Peaks were assigned by spiking the samples with authentic samples of 3-hydroxy-isovaleryl shikonin, acetyl shikonin, and deoxyshikonin, and comparison of the UV-spectra and retention times.

Determination of Embryotoxicity

Male and female rats have been kept in the same cage for 3 days. After this period male rats were displaced from female rat cages. Administration of A-1 was commenced with a single daily oral dose of 100 mg/kg to one of female rat groups on the first day, after which male rats were introduced on the in second day and A-1 continued to be given for seven days. The same dose of A-1 was given to another female rat group immediately after the male rats were placed in the cage. The third and fourth female rat groups received the same treatment, but 48 and 120 hours after male rats were displaced, respectively. The control group received sunflower oil at the same volume as the solubilizing agent. All rat groups were observed for 25 days after the treatment and were kept under observation until the time when they gave birth. The effect of A-1 on the duration of pregnancy and embriological development was compared with that of control. Rats which received A-1 and did not give birth were sacrificed with high dose thiopental sodium (50 mg/kg). Histopathological examination was performed on ovarian and uterine tissue specimens and the results were compared with those in control rats.

Histopathological analyses

Tissue specimens were fixed with 10% buffered formalin and, cross-sections 3 to 5 mm thick were prepared. Tissues were passed through an alcohol series, treated with xylol, and embedded into paraffin. 3 to 5 μ thick tissue slices were then prepared and examined under a light microscope (Olympus BX50, Japan) after staining with hematoxylin-eosin (Magnification Detail: H&E x 40).

RESULTS

Instrumental analysis

Chromatogram of A-1 extract was shown in Figure 2. Relative contents of the acetyl shikonin, deoxyshikonin and 3-hydroxy-isovaleryl shikonin in the extracts were 23.82%, 3.63% and 2.53%, respectively.

Embryotoxicity test

In the control group of rats that was given sunflower oil only, and the group rats which received A-1 (100 mg/kg) 120 hours after male rats displaced (4th group, normal parturition between 22 and 26 days was observed. No birth occurred in other rat groups (1st, 2nd and 3rd groups) that received 100 mg/kg A-1. In 2nd and 3rd groups rats slight bleeding was occurred within 24-48 hours after A-1 administration.

Results of histopathological examinations

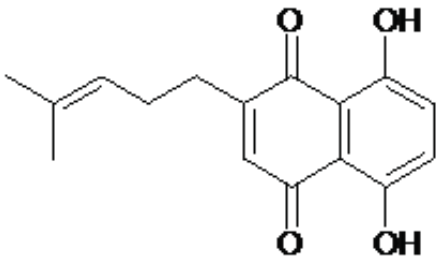
Histopathological examination on uterine and ovarian tissue samples revealed no differences between control rats and rats which received A-1 (Figure 3).

Both groups had primary, secondary and tertiary folliculi in ovarian tissue preparations.

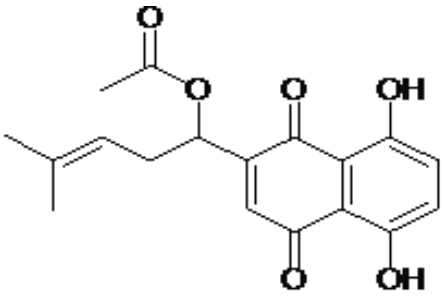
In group A-1, an increase in PNL infiltration, predominantly eosinophilic in nature was noted in endometrial

samples, but with no significant difference compared to the increase observed in the control group (Figure 4).

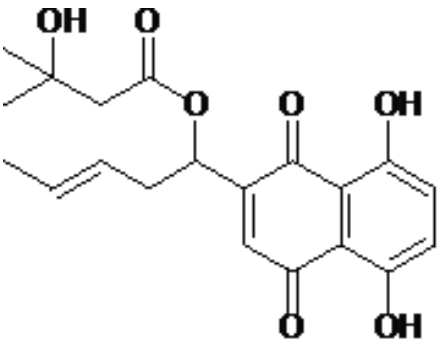
No signs related to presence of an embryo or pregnancy was observed in any rats in A-1 group.



a) Acetyl shikonin



b) Deoxyshikonin



c) 3-Hydroxy-isovaleryl shikonin

Figure 1: Chemical structures of a) Acetyl shikonin b) Deoxyshikonin c) 3-Hydroxy-isovaleryl shikonin

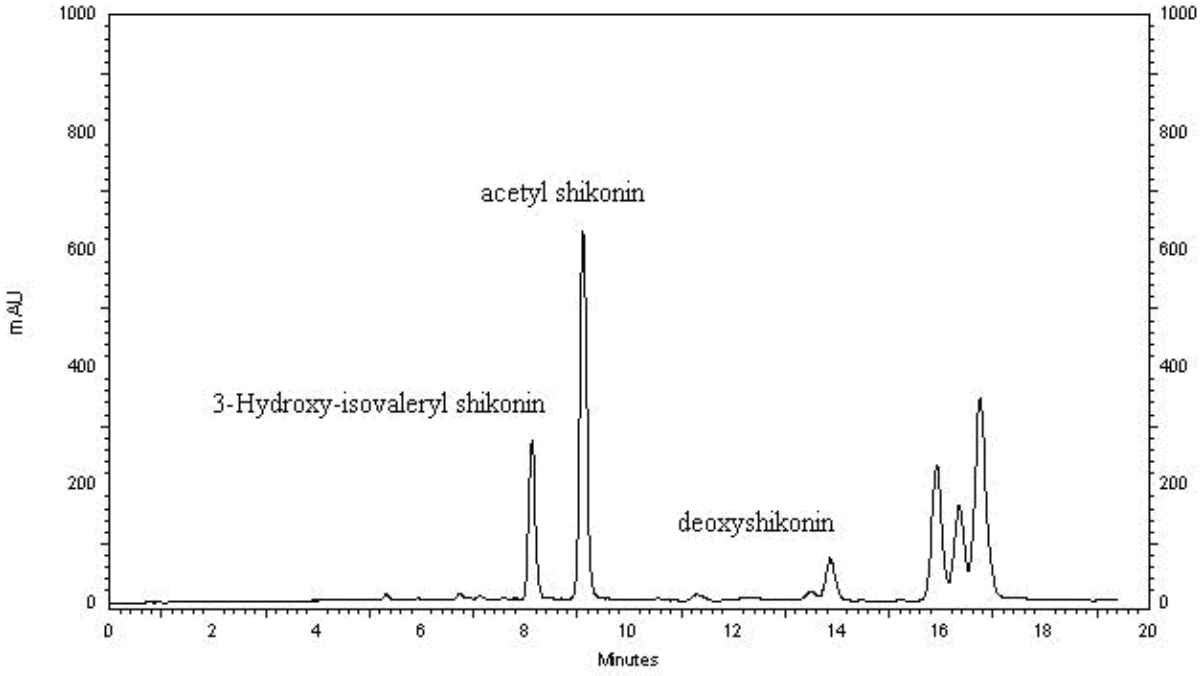


Figure 2: Chromatogram of O. armeniacum extract

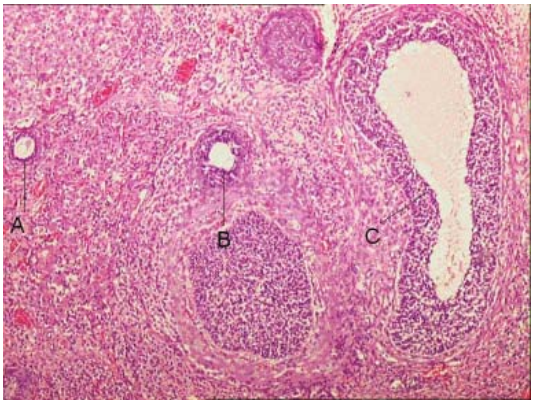


Figure 3: It shows preantral A , antral B and Graafian C follicle of ovary H&E x 40 .

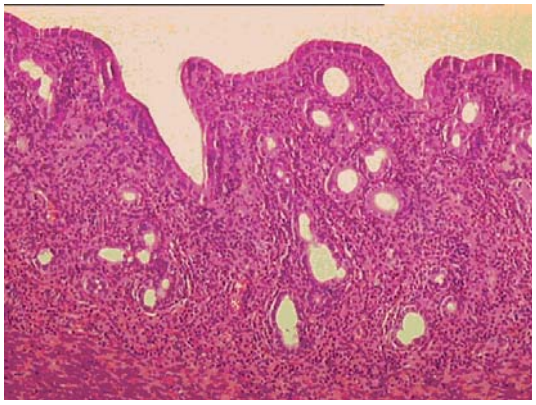


Figure 4: Numerous neutrophils, eosinophils in addition to lymphocytes are surface of the endometrium, glands, and stroma H&E x 40 .

DISCUSSION

In this study, the effects of A-1 extract obtained from the roots of *Onosma armeniacum*, an herbal medicine used as a folk remedy, on the duration of pregnancy and embryonic development in rats were investigated.

As it is known, the periods of pregnancy include pre-implantation, implantation, early post-implantation, organogenesis and fetal periods (Loose and Stancel 2006). In rats, pre-implantation period is 3 to 4 days after fertilization, and the implantation is 5 to 6 days after fertilization (Kayaalp 2002). Our results showed that A-1 prevented the pre-implantation (blastocyst formation) and implantation periods. Of the 10 rats which received A-1 during later stages of pregnancy (9 days after fertilization), the pregnancy resulted in normal labour in 8, suggesting that A-1 has no embryotoxic effects. In pregnant rats, the critical developmental period includes the 7th, 8th and 9th days of the pregnancy (Kayaalp 2002). In this study, the date of fertilization was calculated starting from the time point at which male and female rats were placed in the same cage. In humans, pre-implantation and implantation occur 5 to 8 and 8 to 13 days after fertilization, respectively (Navot et al. 1991); thus A-1 should never be used in the first of months of pregnancy. To elucidate the cause of infertility induced by A-1, histopathological examination was performed on uterus and ovarian tissue preparations obtained from rats that became infertile upon the use of A-1. This revealed no apparent cause for infertility in the tissue samples examined. It seems that A-1 has a contraceptive effect rather than an embryotoxic effect.

The preventive effects of A-1 on pre-implantation and implantation periods is considered to not to relate the toxicity. Such that; there was no sign of acute toxicity or death in the rats that received 500, 1000 mg/kg and higher doses of A-1. In a previous study Arnebia euchroma, a plant from Boraginaceae family which contains shikonins as *Onosma armeniacum*, found to exert contraceptive effect (Findley 1981). Both in vitro and in vivo experimental studies have also shown that *Onosma armeniacum* root extract scavenges toxic oxygen radicals and stimulates antioxidant mechanisms (Cadirci et al. 2007). In addition, in vitro experimental studies have reported that *O. argentatum*, another Onosma species, root extract is a potent antioxidant agent (Ozgen et al. 2003). These results show that A-1 is a non-toxic extract with contraceptive properties.

Conclusion

As a conclusion we can say that A-1 is a nontoxic extract with contraceptive effects rather than embryotoxic effects. Shikonin content of A-1 can be responsible for this contraceptive effect. Further clinical and pre-clinical studies are necessary to characterize these effects of A-1. This study points to the importance of investigating the effects of 150 and more Onosma species, and other folk remedies on all periods of pregnancy.

Acknowledgment

We would like to express our thanks to Prof Dr. Peter J. Houghton and Associated Prof. Dr. Bunyamin Borekci for their contribution to this work.

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