

**STUDY OF EFFECTS OF PINE NEEDLE EXTRACT ON MICROSTRUCTURE OF PLACENTA IN PREGNANT MICE AND SOME OTHER COMPLICATIONS**

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**Summary**

Herb medication was common in ancient Iran and has continued yet . As chemical drugs, Some herbs have side effects too. Nevertheless only destructive effects of some herbs have known yet. Pine needle is a herb medication which is used as abortive agent in some communities. Is it abortive agent really? Has it other destructive effects? These questions induced us to research.

Sixteen female BALB/C mice were divided in control 1, control 2 and experimental groups. They expose to males mice for mating and pregnancy. Vaginal plaque was marked as zero day of gestation. Medication was begun from the first day of gestation and continued to end of pregnancy. Experimental mice received pine needle extract and control 1 mice received only normal saline through gavages. Control 2 group received nothing. Animals were cared and every abortion or death were recorded.

We observed no abnormality in control groups, but all of mice were aborted in experimental group. Fifteen died at time of abortion. Histological evaluation of uterus and placenta af these mice, showed abnormal changes, such as blood congestion, inflammatory cells infiltration, atrophy and necrosis in placenta.

Other investigators had demonstrated mother death, abortion and histological abnormal changes by pine needle consumption. In this research, death, abortion and histological abnormal changes were recorded too. By comparison of these two results we can conclude that pine needle extract may has destructive effects on mother and fetuses.

**Key words:** pine needle; fetus; placenta; abortion.

### **Introduction**

Consumption of boiled extract of pine needle has been used for abortion in Iranian women from several years ago. This nonscientific procedure is used in some regions yet. By some investigations it has been revealed that consumption of pine needle has incursive and destructive side effects on some organs of the body. Eating the pine needle by pregnant cows induced abortion in last trimester(1-4) . It had not destructive effect on fetuses in vitro. Some other investigations revealed that pine needle has toxic effects on organs of the body in addition to abortion (5-10) . Because of rules and ethical importance of this subject and probable toxic effects of pine needle, we decided to study the probable destructive and teratogenic effects of pine needle on fetuses and abortion.

### **Materials and methods**

Sixty female BALB/C mice with two months old and 30 -35 grams weight were divided to three groups randomly . experimental , control1 and control2 were located in five mice boxes. We placed one similar male in each box. vaginal plaque existence was recorded as zero day of pregnancy. Daily gavage of pine needle extract to experimental group and normal saline to control1 group was begun from first day of pregnancy. Quantity and quality of daily Gavage of extract and normal saline to experimental and control1 group respectively were identical. The time of gavage was at 10 o'clock of every day. Control2 group received neither extract nor normal saline. Notice that we cut the gavage after abortion. Amount of gavage was 1.2 mg/kgbw for every day (3) . We had prepared the nonalcoholic extract by an extractor in Mashhad university of medical sciences. Mice were cared with scrutiny during the period of pregnancy and every changes such as abortion were recorded. Aborted fetuses were examined for probability of congenital abnormalities. After macroscopic examination , fetal tissues exposed to preparation for H& Estaining. For this purpose uteruses and placentas were located in 10% formalin for 72 hours. Then dehydration and other tissue processing were done and finally 7 micrometer thick sections from parafinized tissues were dyed by H@E staining. 100 sections from uteruses and 100 from placentas were observed in each group. Observation of specimens have been done by 3 person using Olympus microscope. Existence and inexistence of any changes were recorded. Placental and utrine histological changes were recorded. 100 sections from each group , that is 300 for all groups were observed by Olympus microscope. Half of all sections were uterine and other half were placental. Existence or inexistence of inflammatory cells infiltration , blood congestion and necrosis were recorded in all of groups and compared with each others. Abortions were compared too. Finally statistical analysis was taken place by ANOVA test.

### **Results**

No abortion was observed in control groups. Inflammatory cells infiltration, blood congestion and necrosis were not observed in control groups too (figs.1,3). In addition, we did not observe any abnormal histologic changes in these groups. But all of the mice in experimental group had abortion during the different days of pregnancy. Abortions were begun from day 15 and continued. At day 15 we recorded only one abortion that mother died two days later . At day 16 four abortions were recorded. These mothers died, one at next day and three others at another next day . At fifteenth day we observed 5 abortions. All of these mother died two days after abortion.

At eighteenth day, 5 pregnant mice died and 5 others aborted . These last mice died 6 hours later. Sections which were observed from control groups had not any abnormal changes. But in experimal group ,80 from 100 sections had mixed inflammatory cells infiltration in placental tissue (fig.4).

85 sections from above sessions had blood congestion (fig.4). Finally we observed cell necrosis in 70 sections from 100 above sections (fig.4). In uterine sections, we observe inflammatory cells infiltration in 80 from 100 all sections. Blood congestion in 90 and cell necrosis in75 sections were other our findings (fig.2). These data are related to experimental group. We did'nt observe any abnormal histological changes in control groups (fig.1).

Cell necrosis	Blood congestion	Cell infiltration	Animal groups
0 sections	0 sections	0 section	Control 1
0 sections	0 sections	0 sections	Control2
70 sections	85 sections	80 sections	experimental

1. Placental tissue changes in case and control groups

Cell necrosis	Blood congestion	Cell infiltration	Animal groups
0 sections	0 sections	0 section	Control 1
0 sections	0 sections	0 sections	Control2
75 sections	90 sections	80 sections	experimental

2. Uterine tissue changes in case and control groups

Died in experimental group	Abortion in experimental group	Died in control groups	Abortion in control groups	Gestational days
0	1	0	0	15
0	4	0	0	16
2	5	0	0	17
18	5	0	0	18
0	0	0	0	19
0	0	0	0	20

3. Comparison of changes in case and control groups from 15 -20 gestational days.



Fig.1: uterine tissue (control group); epithelium (star); basement membrane (yellow arrow); lamina propria (red arrow); myometrium (M); H@E staining; 200\*.

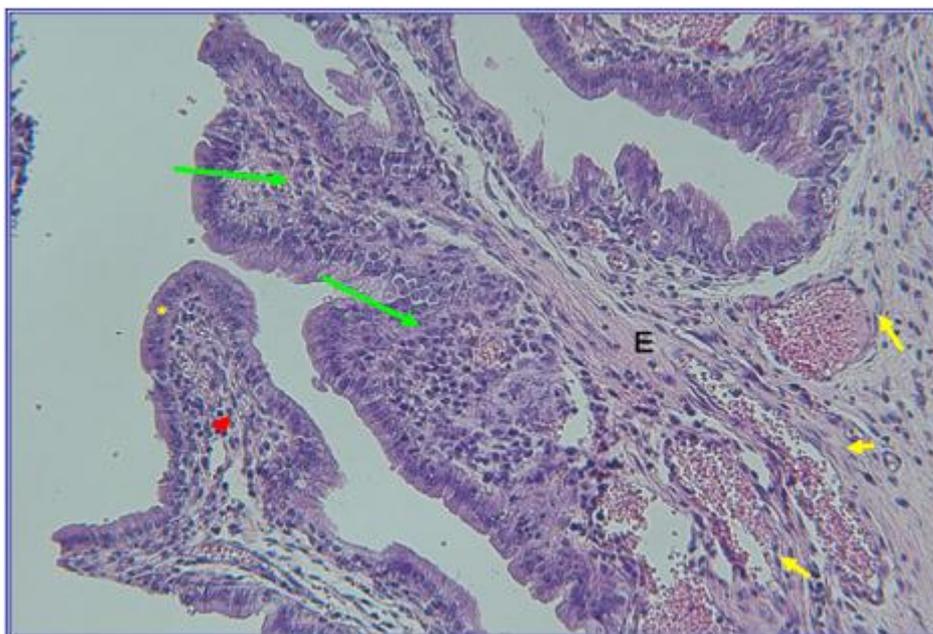


Fig.2: pregnant experimental mouse's uterus (day 15); endometrium (E); epithelium (star); lamina propria (arrowhead); infiltration (green arrow); congestion (yellow arrow); H&E staining; 200\*.

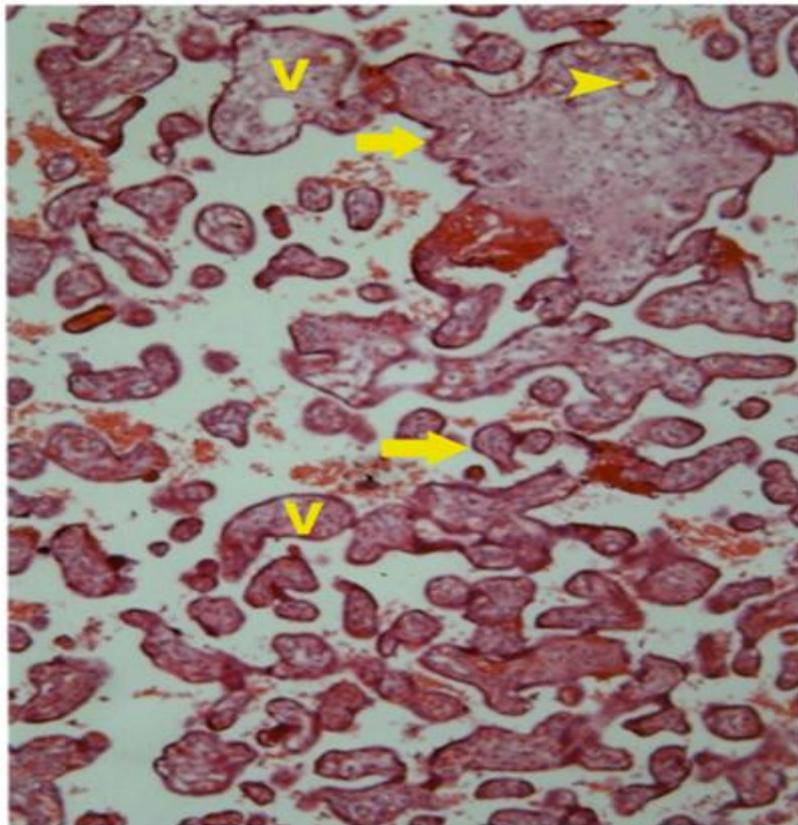


Fig.3: placental tissue (control group); villus (v); cytotrophoblast (arrow); blood vessel (arrowhead); H&E staining; 200\*.

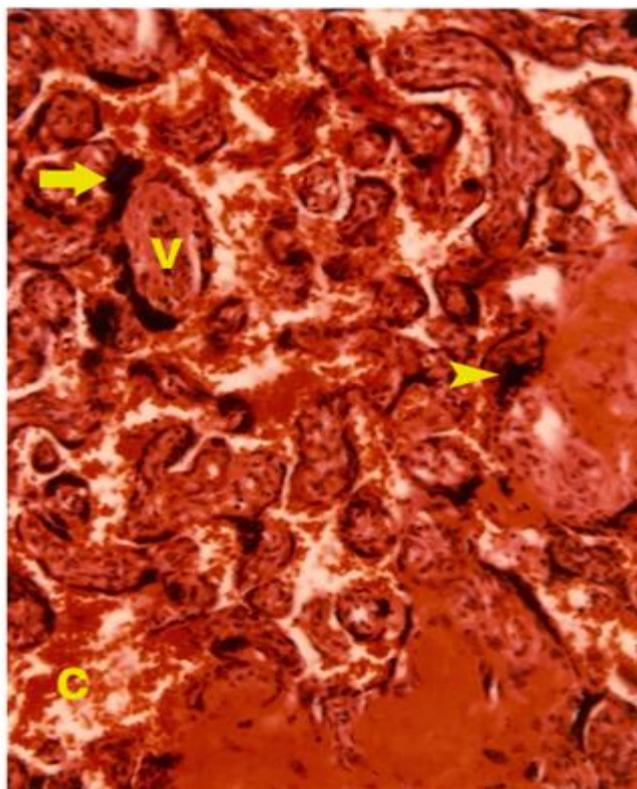


Fig.4: placental tissue (experimental group); villus (v); congestion (c); necrosis (arrow); infiltration (arrowhead); H&E staining; 200\*.

### **Discussion**

There weren't any abortion in both control groups , there weren't any affection of normal saline on controll group . These two results confirmed accuracy of our work. safety of normal saline on pregnant mice, as we used, was confirmed too. Altogether, abortion in experimental group, and comparison of this with control groups , have significant difference statistically ( $P<0.05$ ). comparison of deads between groups showed meaningful differences too. Some other investigators have been reported abortion induced by pine needle too (10-15).

So our results have accommodation with above reports , that confirm ours. No abnormal changes were observed in microstructure evaluation of placenta, at control groups . But some changes such as mixed inflammatory cells infiltration were observed in experimental groups. Comparison of groups from this aspect, shows significant difference. Other abnormal change was blood congestion in placentas of experimental group . inexistence of this phenomenon in control groups have significant difference with experimental group. Another change was cell necrosis in experimental group, comparison of this with control groups results significant difference. All of these results confirm the idea of destructive effects of pine needle on placenta. Some researchers have reported the negative effects of pine needle , such as necrosis , on microscopic structure of placenta(15-17) . So our results were confirmed by other results reported above. We can conclude from all of results of these investigations that consumption of pine needle probably cause abortion in mice. These abortions will occurred by abnormal tissue changes such as mixed inflammatory cells infiltration , blood congestion and necrosis in placenta. We evaluate these changes ultrastructurally too. The results will report later.

### **References**

1. S. Wang ; K. E. Panter ; D. R. Gardner ;R. C. Evans and T. D. Bunch. Effects of the pine needle▶ abortifacient, isocupressic acid, on bovine oocyte maturation and preimplantation embryo development. *Anima reproduction science*; volume 81, issue 3-4, april 2004,page 237-244.
2. W. D. Wagner and L. L. Jackson . Phytoestrogen from assayed by competitive binding with  $17\beta$ -estradiol to mouse uterine cytosol. *theriogenology*, volume 19, issue 4, april 1983, pages 507-516.
3. Gardner DR, Panter KE, James LF, Stegelmeier BL. Abortifacient effects of lodgepole pine (*Pinus contorta*) and common juniper (*Juniperus communis*) on cattle. *Vet Hum Toxicol*. 1998 Oct;40(5):260-3.
4. Stegelmeier BL, Gardner DR, James LF, Panter KE, Molyneux RJ. The toxic and abortifacient effects of ponderosa pine. *Vet Pathol*. 1996 Jan;33(1):22-8.
5. Short RE, Ford SP, Grings EE, Kronberg SL. Abortifacient response and plasma vasoconstrictive activity after feeding needles from ponderosa pine trees to cattle and sheep. *J Anim Sci*. 1995 Jul;73(7):2102-4.

6. Short RE, Staigmiller RB, Bellows RA, Ford SP. Endocrine responses in cows fed Ponderosa pine needles and the effects of stress, corpus luteum regression, progestin, and ketoprofen. *J Anim Sci.* 1995 Jan;73(1):198-205.
7. Bavister, B.D., 1995. Culture of preimplantion embryos: facts and artifacts. *Hum. Reprod. Update* 1 2, pp. 91–148.
8. Bavister, B.D., Rose-Hellekant, T.A. and Pinyopummintr, T., 1992. Development of in vitro matured in vitro fertilized bovine embryos into morulae and blastocysts in defined culture media. *Theriogenology* 37, pp. 127–146.
9. Cetica, P.D., Dalvit, G.C. and Beconi, M.T., 1999. Study of evaluation criteria used for in vitro bovine oocyte selection and maturation. *Biocell* 23 2, pp. 125–133.
- 10 Ford, S.P., Rosazza, J.P.N., Al-Mahmoud, M.S., Lin, S., Farley, D.B. and Short, R.E., 1999. Abortifacient effects of a unique class of vasoactive lipids from *Pinus ponderosa* needles. *J. Anim. Sci.* 77 8, pp. 2187–2193
11. Gardner, D.R., Molyneux, R.J., James, L.F., Panter, K.E. and Stegelmeier, B.L., 1994. Ponderosa pine needle-induced abortion in beef cattle: identification of isocupressic acid as the principal active compound. *J. Agric. Food Chem.* 42 3, pp. 756–761.
12. Gardner, D.R., Panter, K.E., Molyneux, R.J., James, L.F. and Stegelmeier, B.L., 1996. Abortifacient activity in beef cattle of acetyl- and succinylisocupressic acid from ponderosa pine. *J. Agric. Food Chem.* 44 10, pp. 3257–3261.
13. Gardner, D.R., Panter, K.E., James, L.F. and Stegelmeier, B.L., 1998. Abortifacient effects of lodgepole pine (*Pinus contorta*) and common juniper (*Juniperus communis*) on cattle. *Vet. Hum. Toxicol.* 40 5, pp. 260–263.
14. Gardner, D.R., Panter, K.E. and James, L.F., 1999. Pine needle abortion in cattle: metabolism of isocupressic acid. *J. Agric. Food Chem.* 47 7, pp. 2891–2897.
15. Hawk, H.W. and Wall, R.J., 1994. Improved yields of bovine blastocysts from in vitro-produced oocytes. I. Selection of oocytes and zygotes. *Theriogenology* 41 8, pp. 1571–1583.
16. McEvoy, T.G., Robinson, J.J., Ashworth, C.J., Rooke, J.A. and Sinclair, K.D., 2001. Feed and forage toxicants affecting embryo survival and fetal development. *Theriogenology* 55 1, pp. 113–129.
17. Wang, S., Liu, Y., Holyoak, G.R. and Bunch, T.D., 1997. The influence of M-199 and a modified CR-2 medium on the in vitro production of bovine embryos. *Theriogenology* 47, p. 304.