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The Effects of Essential Oil of Galbanum on Caffeine Induced-Cleft palate in Rat Embryos

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Abstract

Background: Caffeine at high doses is a known rodent teratogen and induces limb malformations along with cleft palate in various strains of rats and mice. The teratogenic effects of some drugs can be prevented by the application of antioxidant drugs and stimulation of the maternal immune system. Also, there is some evidence that galbanum is antioxidant. Therefore, in this study, the prophylactic effect of galbanum on teratogenic effects of caffeine was evaluated.

Materials and Methods: This experimental study was performed on 28 pregnant rats that were divided into four groups. Control group received normal saline and test groups received caffeine (80 mg/kg), caffeine (80 mg/kg) plus galbanum (200 mg/kg) and galbanum (200 mg/kg), intraperitonealy at 9-11th days of gestation, respectively. Fetuses were collected at 20th day of gestation and after determination of weight and length; they were stained by Alizarin red - Alcian blue method.

Results: Cleft palate incidence was 33.3%, in caffeine group and decreased to 8.3% by galbanum. The mean of weight and length of fetuses from rat that received galbanum were significantly greater than those received only caffeine.

Conclusion: It is concluded that galbanum decreased cleft palate induced by caffeine; but its mechanism needs more details evaluation.

Introduction

Maternal exposure to caffeine (as drug or coffee consumption) can be affect fetus [1, 2]. Caffeine at high doses is a known rodent teratogen and induces limb malformations along with cleft palate in various strains of rats and mice [3]. Several studies have demonstrated the teratogenicity of caffeine in laboratory animals [4-6], experimental results cannot be applied to humans because to the variability of caffeine dose, exposure time and species differences.

The sensitivity of different animal's species is variable. Malformations have been demonstrated in mice at 50-75 mg/kg of caffeine, whereas the lowest dose usually needed to induce malformations is 80 mg/kg in rats. However, when caffeine is administered in fractioned amounts during the day, 330 mg/kg/day are necessary to reach teratogenicity in rats [7]. In rodents, the most frequently observed malformations are those of the limbs and digits, ectrodactyly, craniofacial malformations (labial and palatal clefts) and delays in ossification of limbs, jaw and sternum. Nevertheless, even in rodents, caffeine can be considered as a weak teratogenic agent; given the quite large quantities of caffeine necessary to induce malformations and the small number of animals affected [7].

Caffeine potentiates the teratogenic effect of other substances, such as tobacco, alcohol, and acts synergistically with ergotamine and propranolol to induce maternofetal vasoconstrictions leading to malformations induced by ischemia. Therefore, even though caffeine does not seem to be harmful to the human fetus when intake is moderate and spread out over the day, some associations, especially with alcohol, tobacco, and vasoconstrictive or anti-migraine medications should be avoided. Maternal exposure to caffeine induces long-term consequences on sleep, locomotion, learning abilities, motivity, and anxiety in rat offspring, whereas in humans, more studies are needed to ascertain long-term behavioral effects of caffeine ingestion by pregnant mothers [7].

Some of these caffeine-derived effects could favour the production of free radicals and a subsequent increase of oxidative stress such as the metabolic inactivation of catecholamines [8] and the increase of oxidative metabolism including its own hepatic metabolism [9]. There are also reports suggesting that caffeine is capable of including certain forms of oxidative damage by increasing lipid peroxidation [10]. *Ferula gummosa* Boiss, a monocarpic plant from Umbelliferae family, is prized for its oleogum called galbanum, a mixture of essential oil and resin that is produced in the tuber of this perennial plant. Nomads of Southwest Iran call this plant 'Barijeh' and traditionally use its resin for the treatment of diarrhea [11]. For a long time galbanum oil was used for different medicinal and spiritual purposes and, as written in the book of Exodus (30:34), it was the favourite

oil of Moses. In Iranian ancient medicine, the gum obtained from the aerial parts of this plant has been used as antiseptic, antispasmodic, anti inflammatory, wound-healing remedy and antitoxic in the past [12]. Today, *Ferula gummosa* is recognized for its antibacterial and health promoting properties. Many essential oil compounds of galbanum are very important for medicinal [11-13] industrial and perfumery uses. The major habitat of *Ferula gummosa* is high altitude mountains of Iran and this country is the most important exporter of galbanum gum.

An antinociceptive activity has been shown for the hydroalcolic extract of aerial parts of *Ferula gummosa* [14] and acetone extract of Ferula gummosa seed and root has been reported previously [15]. Furthermore, a methanol-chloroform extract of *Ferula gummosa* and its fractions have alleviated the morphin withdrawal syndrome induced by naloxane [12]. Dehpour et al. reported good antioxidant activity from a methanol extract of Ferula assafoetida, another species from the ferula genus [16]. Nabavi et al. reported that hydroalcolic extract of *Ferula gummosa* Bioss flowers, stems and leaves had remarkable antioxidant and antihemolytic effect that maybe results of its high phenol and flavonoid contents [17]. In present study, the preventive effect of galbanum on caffeine-induced cleft palate in rats was evaluated.

Materials and Methods

This experimental study was done in animal model in department of basic sciences of faculty of veterinary medicine of Shahid Chamram University (Ahvaz-Iran). The animal care was provided under the supervision of a qualified veterinarian. Caffeine powder (Merck, Germany) and essential oil of galbanum (Barij Essence Pharmaceutical Company, Kashan, Iran) were purchased. Male and female healthy rats of Wistar strain, 3-4 month old, weighing 200-250 g were purchased (Joundishapour laboratory animal center, Ahvaz, Iran) and housed individually (males) or at 10 per polycarbonate cage (female) for a 2-week acclimation period. Rats were fed ad libitum by standard laboratory pellet (Pars Khurakdam, Tehran, Iran) and tap water. A 12 h light: 12 h dark was mentioned. Room temperature was at 23±2ºC with a relative humidity of 45-55%. Females were mated overnight with males. Pregnancy was ascertained the next morning by presence of a vaginal plug, and this time was designated as gestational day (GD) 0.

Drug administration: Pregnant rats (N=28) were randomly divided into four groups (20 pregnant rats in treatment groups, 8 pregnant rats in control group) and treated as follow:

Group 1: Control group: normal saline in equal volume of caffeine was injected to pregnant rats for inducing similar condition (injection and handling) to other groups. Group 2: Caffeine group: caffeine (80 mg/kg) was administrated intraperitonealy at 9-11th day of gestation. Group 3: Galbanum group: galbanum (200 mg/kg) was administrated intraperitonealy at 9-11th day of gestation. Group 4: caffeine + galbanum group: caffeine (80 mg/kg) plus galbanum (200 mg/kg) was administrated intraperitonealy at 9-11th day of gestation.

Sampling and staining: The animals were sacrificed by euthanized and cervical dislocation at 20th day of gestation. Following laparotomy, the uterus was exteriorized and the number and location of fetuses and resorption were noted, then their weight and length (crown- rump length) were measured. Individual fetuses were examined carefully for external anomalies then fetuses were stained by Alizarin red-Alcian blue method [18] and investigated by stereomicroscope (Nikon, SMZ200, Japan) for skeletal malformations. Then the placenta were weighed, measured, and examined macroscopically. The incidence of skeletal malformations was determined and was compared between groups.

Statistical: Statistical significance between groups was determined using SPSS-16 program and compared by one way analysis of variance (ANOVA) and Post hoc LSD. The minimum level of significance was $p<0.05$.

Results

Sixty two fetuses were obtained from eight rats of control group. There were not observed macroscopic anomalies in the control animals. In the control group palatal closures of fetuses were normal at gestational day 20 (i.e., palatal shelves had grown vertically on the sides of the tongue, then horizontally to meet and fuse). No maternal death or abortion occurred in any experimental groups. There were not any aborted fetuses from total groups but percentage of resorbed fetuses were 0%, 3.38%, 0%, and 0% in groups that received normal saline, caffeine, caffeine plus galbanum and galbanum, respectively, so galbanum decreased resorption rate (Table 1). Caffeine induced cleft palate (Fig. 1B) at 33.33% incidence. Caffeine plus galbanum (200 mg/kg) significantly reduced incidence of cleft palate to 8.3%.

The mean of weight of animals' fetuses that received caffeine (80 mg/kg) in 9-11th days was significantly decreased in comparison with other groups (Table 1). The mean of weight and length of animals' fetuses that received galbanum in 9-11th days was significantly decreased in comparison with control group (Table 1).

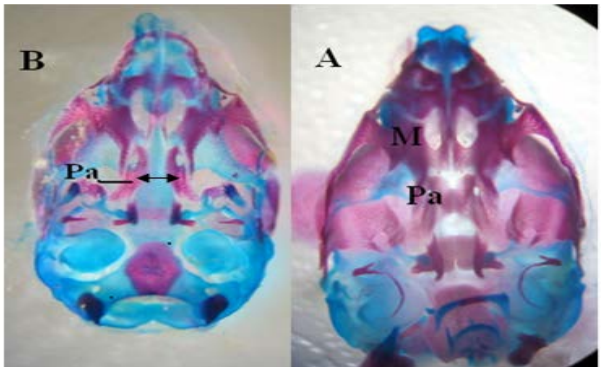

Figure 1. Ventral view of skull of rat fetuses of GD 20, stained with alizarin red S- alcian blue. A) Normal palatine bone B) Cleft palate induced by caffeine (arrow). M: maxilla; Pa: palatine

Table 1. Incidence of anomalies in rat fetuses of groups

%fetuses with cleft palate	Fetal weight (g) (mea n±SEM)	Fetal length (mm) (mea n±SEM)	Live fetuses	Resorbed fetuses	Implantations	No. of litters	Groups
0(0)	4.93±0.08*	38.01±0.26*	62	0(0)	62	8	Control
19(33.33)#	3.032±0.17**	28.92±0.81**	57	2(3.38)	59	6	Caffeine
5(8.33)	3.75±0.11	34.97±0.45	60	0(0)	60	7	Caffeine+ Galbanuml
0(0)	4.06±0.10	35.403±0.26	57	0(0)	57	7	Galbanum

Numerals in parantheses are percentages,* Significant difference with other groups (p<0.05), ** Significant difference with other groups (p = 0.0001), #: Incidence of cleft palate was significantly difference at groups which received caffeine with control and galbanum groups (p = 0.0001).

Discussion

We demonstrated caffeine (at dose 80 mg/kg, IP) decreased weight and length and produced cleft palate (33.3% present of fetuses). Caffeine possesses the potential to derange the processes involved in cell proliferation. Because it has been known for some time that caffeine readily crosses the placenta and reaches the fetus [19], the warning of the food and Drug Administration merits serious consideration.

According to a report by Lelo et al. the average daily human caffeine intake of moderate to heavy consumers ranges from approximately 300 to 600 mg/kg/day, or from 3 to 6 cups of coffee (assuming 100 mg/cup). The dosage level therefore in a person weighing 70 kg ranges from approximately 4.3 to 8.6 mg/kg/day [20]. In comparison, caffeine was administrated to laboratory animals ranged from 30 mg/kg [6] to 250 mg/kg [4]. Even when species variation is taken into account, the practical application of the results obtained from many of these animal experiments to the human condition is unrealistic due to the excessive dose levels administered [7].

A moderate dosage level of 80 mg/kg caffeine was administered as a three intraperitoneal injection on gestational days 9-11th. Fujii et al demonstrated that in mice, whereas embryolethality is related to the duration of caffeine exposure, teratogenic effects are more dependent on a sufficiently high concentration of the drug [5].

Fujii and Nishimura postulated that caffeine was teratogenic by virtue of catecholamine release from maternal or embryonic tissue [4]. Moriguchi and Scott (1986) reported that administering 175 mg/kg of caffeine intraperitonealy at 1600 h day 11 and 900 h day 12 in mice induced malformation that is initiated by release of catecholamines from the maternal adrenal gland [3].

Ross et al. reported neural tube defects in early rat embryos following maternal treatment with caffeine. Significant resorptions by caffeine to pregnant rats at a dose level of 120 mg/kg on the day 12 of gestation [21].

In present study, embryo from mothers treated with caffeine revealed a significant reduction in crown-rump length. It is believed that maternal treatment with caffeine alters utero-placental circulation to such an extent that normal embryonic development is impaired [22]. Burdan reported that the mixture of paracetamol and caffeine decreased fetal length and body weight, and placental weight [23]. Nishimura and Nakai reported increased cleft palate and digital defects in mice off spring exposed to caffeine at a dose of 250 mg/kg [24].

A lack of embryo – or fetotoxicity or teratogenicity was observed when caffeine was administrated for whole gestational period at doses 16-17 and 25-33 per day [25]. A reduction in fetal weight was found after maternal pregnancy exposure ton 62 mg/kg per day. In contrast, Nolen reported that daily, long-term caffeine exposure at doses up to 80 mg/kg per day in drinking water did not affect fetal development. They also showed that such administration caused no differences in body weight gain or feed consumption [26, 27]. Aeschbacher et al. reported that caffeine dietary concentration of 0.25 and 0.5 g/kg throughout gestation and lactation had no significant on birth weight, litter size or development [24]. However, fetal loss, decreased fetal weight and size, and major skeletal defects have been reported when dosages of more than 80 mg/kg of caffeine were used [28].

Since there are not data available on galbanum on the teratogenicity of caffeine in rat embryos. Several studies have reported that the maternal immune stimulation can reduce teratogenic anomalies [29]. Mechanisms of this effect remain unclear, but it is thought the fetal gene expression has been modulated [30].

The enhancing antioxidative effects can protect fetuses against drugs teratogenicity [31]. Sharova et al. showed that interferon-gamma and Freund's complete adjuvant reduced severity of the urethane-induced cleft palate in mice [32].

A number of observation suggest that detoxification of a xenobiotic free radical intermediate with antioxidants may provide important embryoprotection [32]. Nabavi et al. reported that hydroalcolic extract of *Ferula gummosa* Bioss flowers, stems and leaves had remarkable antioxidant and antihemolytic effect that maybe results of its high phenol and flavonoid contents [17]. Dehpour et al. reported good antioxidant activity from a methanol extract of Ferula assafoetida, another species from the ferula genus [16]. *Ferula gummosa* improves antioxidant defenses and has cardioprotective effect [33]. Also, its root extract has antioxidant and antihemolytic activities which related to phenols and flavonoids in the extract [34]. In conclusion, the present study showed the effects of galbanum for the first time on cleft palate induced caffeine in rat fetuses. The present results indicate that exposure 80 mg/kg of caffeine in 9-11th days of gestation of rat decreases weight and length of embryos and did influence on skeletal system. It is probably caffeine influences antioxidant system that produces teratogenic effects including cleft palate. Effects of caffeine immunosuppression are mediated indirectly by inducing

oxidative stress. The protective effect of galbanum in caffeine-induces cleft palate in rat may, at least in part, be due to its antioxidant activity, which we believe deserves further investigation.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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References

1. Olcina GJ, Munoz D, Timon R. Effect of caffeine of on oxidative stress during maximum incremental exercise. J Sport Sci Med 2006; 5(4): 621-628.
2. Nash JE, Persaud TV. lnfluence of nicotine and caffeine on rat embryonic development. Histol Histopath 1988; 3(4): 377-388.
3. Moriguchi M, Scott WG Jr. Prevention of caffeine-induced limb malformations by maternal adrenalectomy. Teratology 1996; 33(3): 319-322.
4. Fujii, T, Nishimura H. Teratogenic actions of some methylated xanthines in mice. Okajimas Folia Anat Jap 1969; 46(4): 167-175.
5. Fujii T, Sasaki H, Nishimura H. Teratogenicity of caffeine in mice related to its mode of administration. Jpn J Pharmacol 1969; 19(1): 134-138.
6. Palm PE, Arnold EP, Rachwall PC, et al. Evaluation of the teratogenic potential of fresh-brewed coffee and caffeine in the rat. Toxicol App Pharmacol 1978; 44(1): 1-16.
7. Nehlig A, Debry G. Potential teratogenic and neurodevelopmental consequences of coffee and caffeine exposure: A review on human and animal data. Neurotoxicol Teratol 1994; 16(6): 531-43.
8. Jewett SL, Eddy LJ, Hochstein P. Is the autoxidation of catecholamines involved in ischemia-reperfusion injury? Free Radic Biol Med 1989; 6(2): 185-188.
9. Vistisen K, Poulsen HE, Loft S. Foreign compound metabolism capacity in man measured from metabolites of diatery caffeine. Carcinogenesis 1992; 13(9): 1561-8.
10. Dianzani MU, Muzio G, Biocca ME and Canuto RA. Lipid peroxidation in fatty liver induced by caffeine in rats. Int J Tissue Reac 1991; 13(2): 79-85.
11. Sadraei H, Asghari GR, Hajhashemi V, et al. Spasmolytic activity of essential oil and various extracts of Ferula gummosa Boiss. on ileum contractions. Phytomedicine 2001; 8(5): 370-376.
12. Zargari A. Medicinal Plants. Tehran: Tehran University Publication; 1991: 598-602.
13. Ramezani M, Hosseinzadeh H, Mojtahedi K. Effect of Ferula gummosa Boiss. fractions on morphine dependence in mice. J Ethnopharmacol 2001; 77(1): 71-75.
14. Fazli Bazaz BS, Parsaei H, Harirzadeh G, Shoshtari AN. Evaluation of antinociceptive and antimicrobial activities of galbanum plant (Ferula gummosa). Daru 1997; 7(4): 1-22.
15. Mandegary A, Sayyah M, Heidari MR. Antinociceptive and anti-inflammatory activity of the seed and root extracts of Ferula gummosa Boiss in mice and rat. Daru 2004; 12(2): 58-62.
16. Dehpour AA, Ebrahimzadeh MA, Nabavi SF and Nabavi SM. Antioxidant activity of the methanol extract of Ferula assafoetida and its essential oil composition. Grasa y Aceites 2009; 60(4): 405-412.
17. Nabavi SF, Ebrahimzadeh MA, Nabavi SM and Eslami B. Antioxidant activity flower, stem and leaf extracts of Ferula gummosa Boiss. Grasas y Aceites 2010; 61(3): 244-250.
18. Kimmel CA, Trammekl CA. A rapid procedure for routine double staining of cartilage and bone in fetal and adult animals. Stain Technol 1981; 56(5): 271-273.
19. Goldestein A, Warren R. Passage of caffeine into human gonadal and fetal tissue. Biochem Pharmacol 1962; 11: 166-8.
20. Lelo A, Miners JO, Robson R and Birkett DJ. Quantitative assessment of caffeine partial clearances in man. Br J Clin Pharmacol 1986; 22(2): 183-186.
21. Ross CP, Persaud TV. Neural tube defects in early rat embryos following maternal treatment with ethanol and caffeine. Anat Anz 1986; 169(4): 247-52.
22. Adamson K, Muller-Heubach E, Meyers RE. Production of asphyxia in the Rhesus monkey by administration of catecholamines to the mother. AM J Obested Gynecol 1971; 109(2): 248-262.
23. Burdan F. Intrauterine growth retardation and lack of teratogenic effects of prenatal exposure to the combination of paracetamol and caffeine in wistar rats. Reprod Toxicol 2003; 17(1): 51-58.
24. Nishimura H, Nakai K. Congenital malformation in offspring of mice treated with caffeine. Proc Soc Exp Biol Med 1960; 104: 140-142.
25. Aeschbacher HU, Milon H, Foot A and Wurzner HP. Effect of caffeine on rat offspring from treated dams. Toxicol Lett 1980; 7(1): 71-77.
26. Nolen GA. The effect of brewed and instant coffee on reproduction and teratogenesis in the rats. Toxicol Appl Pharmacol 1981; 58(2): 171-183.
27. Nolen GA. A reproduction/teratology study of brewed and instant decaffeinated coffees. J Toxicol Environ Health 1982; 10(4-5): 769-782.
28. McKim EM. Caffeine and its effects on pregnancy and the neonate. J Nurse Midwifery 1991; 36(4): 226-31.
29. Ivnitsky I, Torchinsky A, Savion S, et al. TGFbeta2 in embryos with inborn anomalies: effect of maternal immunopotentiation. Am J Reprod Immunol 2001; 45(1): 41-51.
30. Holladay SD, Sharova LV, Punareewattana K, et al. Maternal immune stimulation in mice decreases fetal malformations caused by teratogens. Int Immuno Pharmacol 2002; 2(2-3): 325-332.
31. Winn LM, Wells PG. Maternal administration of superoxide dismutase and catalase in phenytoin teratogenicity. Free Radic Biol Med 1999; 26(3-4): 266-274.
32. Sharova LV, Gogal RM, Sharov AA, et al. Stimulation in urethane- exposed pregnant mice increase expression level of spleen leukocyte genes for TGF beta 3 GM- CSF and

other cytokines that may play a role reduced chemical - induced birth defects. Int Immunopharmacol 2002; 2(10): 1477-89.
33. Gholitabar S, Roshan VD. Effect of Treadmill Exercise and Ferula gummosa on Myocardial HSP72, Vascular Function, and Antioxidant Defenses in Spontaneously Hypertensive Rats. Clin Exp Hypertens 2013; 35(5): 347-354.
34. Ebrahimzadeh MA, Nabavi SM, Nabavi SF and Dehpour AA. Antioxidant activity of hydroalcholic extracts of Ferula gummosa Boiss roots. Eur Rev Med Pharmacol Sci 2011; 15(6): 658-64.

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