

Anbarnesa: The Past Tradition, the Future Medicine

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Abstract

Context: In the Iranian traditional medicine, anbarnesa smoke derived from burning female donkey's dung has long been used for treatment of inflammatory ulcers and infections of the middle and external ear with no significant side effects. The aim of this study was to introduce anbarnesa and discuss its therapeutic effects.

Evidence Acquisition: We conducted a systematic search in PubMed, Medline, Google, and Google Scholar databases to find studies on anbarnesa. The keywords searched were as follows: "anbarnesa," "traditional medicine," "medicinal smoke," "donkey," "dung," "antimicrobial," "inflammation," "infection," and "cytotoxicity."

Results: Literature review reveals that ANNAS (anbarnesa smoke) enhances wound healing, decreases scar formation, inhibits growth of cancer cells (Hela and KB) and has antimicrobial properties. Also, ANNAS combined with propylene glycol is nontoxic in 1/64, 1/128, and 1/256 dilutions.

Conclusions: The constituents of anbarnesa smoke mainly possess antibacterial, anti-inflammatory, and growth inhibition effects on cancer cells.

Keywords: Infection, Inflammation, Medicine, Traditional, Anbarnesa, Antimicrobial, Cytotoxicity, Donkey, Dung, Medicinal Smoke

1. Context

Considering the numerous side effects of synthetic drugs and advances in the alternative medicine in recent years, traditional medicine and herbal medications have gained the spotlight and found their way into modern medicine and dentistry (1, 2). Researchers are in search of natural medications with fewer side effects. The Iranian traditional medicine contains valuable information in this respect (3).

In Iranian traditional medicine, anbarnesa smoke derived from burning female donkey's dung collected in spring has long been used for the treatment of inflammatory oral ulcers such as aphthous ulcers and other inflammatory conditions such as infections of the middle and external ear with no significant side effects (3-8). Avicenna used anbarnesa smoke for vaginal infections and decreasing the duration of menstrual period and stanch bleeding (3, 6, 8). Evidence shows that using the smoke of medicinal plants for treatment of diseases is common not only in Iran, but also in over 50 countries (9).

The preliminary research with contemporary standard methodology on the effects of this traditional treatment (anbarnesa smoke) was started in 2010 at Shahid Beheshti University of Medical Sciences by preparing anbarnesa solution and mouthwash (in propylene glycol solvent); the study is still ongoing (3-5).

The aim of this study was to introduce anbarnesa and discuss its therapeutic effects through literature review.

2. Evidence Acquisition

We conducted a systematic search in PubMed, Medline, Google, and Google Scholar databases on anbarnesa smoke. The keywords searched were as follows: "anbarnesa," "traditional medicine," "medicinal smoke," "donkey," "dung," "antimicrobial," "inflammation," "infection," and "cytotoxicity." All the identified abstracts, letters to the editors, review articles, original articles, animal studies, and other documents were reviewed. The publication language was not an exclusion criterion, and studies published from July 1970 to July 2015 were included. Reference lists of the retrieved articles were also reviewed to identify citations that complied with our inclusion criteria. Articles without an accessible full-text version or without scientific methodology were excluded. Eventually, 5 studies (3, 5, 10, 11) were chosen upon complete agreement between the two reviewers.

3. Results

A literature search using the above-mentioned criteria yielded a total of 4 articles on anbarnesa smoke (4, 5, 10, 11). All articles had been conducted by the Iranian researchers. Two articles were in English (4, 5) and 2 in Farsi (10, 11). Detailed findings are presented in Table 1.

Table 1. Summary of 5 Studies on Anbarbarnesa Smoke

Author	Year	Methods	Materials	Conclusion	Study Design
Shafiee et al. (4)	2014	Analysis of anbarbarnesa smoke using a GC-mass device- Cell viability by methyl thiazolyl tetrazolium assay and reading of the results by ELISA Reader	Test group: Anbarbarnesa smoke with propylene glycol solvent (ANNAS) 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, and 1/256 dilutions were prepared. Assessments were done 1, 2, 4 and 72 hours after contact with the cells- Positive and negative (solvent) groups-Cell line: L929 fibroblasts (normal cells)	Anbarbarnesa smoke consists of substances such as hexane, citric acid, and dimethylamine. Anbarbarnesa is nontoxic in 1/64, 1/128, and 1/256 dilutions. In 1/32 dilution, toxicity was seen after 72 hours. In 1/8 and 1/16 dilutions, toxicity was seen in the first hour.	Experimental in vitro study
Sadeghi-Aliabadi et al. (10)	2013	Cell viability by methyl thiazolyl tetrazolium assay and reading of the results by ELISA Reader	Test group: Anbarbarnesa smoke combined with n-hexane and water solvent. Original and 0.2, 0.175, 0.15, 0.125, and 0.1 mg/mL concentrations were prepared. Assessments were done after 48 hours- Positive and negative (solvent) groups-Cell line: epidermoid Hela (cancer cell), epidermoid KB (cancer cell) an L929 fibroblast (normal cell)	Anbarbarnesa smoke was toxic in 2 mg/mL concentration on Hela and KB cells and was nontoxic in higher concentrations on L929 fibroblasts.	Experimental in vitro study
Anaraki Firouz et al. (3)	2011	Histological test: number of myofibroblasts in superficial and deep areas of wounds	Test group: ANNAS-Control group: propylene glycol wounds were created on the back of the neck of 16 Wistar rats (two wounds on each rat: one test and one control). Assessments were made after 14 and 21 days.	Number of myofibroblasts decreased by 3 folds following the application of ANNAS on superficial and deep areas of wounds after 14 and 21 days. Hair follicles and sebaceous glands appeared after 21 days	Experimental animal study
Shafiee et al. (5)	2012	Growth inhibition zone diameter and minimum inhibitory concentration	Test group 1: 0.2% ANNAS Test group 2: 0.2% chlorhexidine Control group: Propylene glycol Microorganisms: <i>Streptococcus mutans</i> , <i>Streptococcus salivarius</i> , <i>Streptococcus sanguinis</i> , <i>Streptococcus pyogenes</i>	0.2% ANNAS mouth rinse had antibacterial properties; but it was not as efficacious as 0.2% chlorhexidine mouthwash on <i>Enterococcus faecalis</i>	Experimental in vitro study
Parvin et al. (11)	2010	Growth inhibition zone diameter	Test group 1: Direct contact with anbarbarnesa smoke Test group 2: Peganum harmala smoke Control group: Antibiotic negative control group: Straw smoke Microorganisms: <i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i>	Anbarbarnesa smoke was effective on <i>Pseudomonas aeruginosa</i> and <i>Staphylococcus aureus</i> . Peganum harmala smoke was effective only on <i>Staphylococcus aureus</i> .	Experimental in vitro study

Also, 3 doctoral (DDS) theses had been conducted on anbarbarnesa smoke under the supervision of Dr H. Shafiee (3, 12, 13). Of them, 2 had been converted to Eng-

lish articles (4, 5, 12, 13) and the remaining one was conducted on Wistar rats (3) (Table 1).

4. Conclusions

Shafiee et al. (4) analyzed the smoke from burning of anbarnesa using chromatography by GC mass device and isolated its constituents as hexane, acetic acid, aconitane, beta carotene, dimethyl amine. These compounds mainly have antibacterial, antifungal, anti-inflammatory and antioxidant properties and some of them are used for treatment of neuralgia, rheumatism, capillary hemorrhage, and skin disorders (3, 4, 14).

An in vitro study showed that a mouthwash (0.2% ANNAS SBMU 1) prepared from anbarnesa smoke had antimicrobial and bacteriostatic properties and yielded a growth Inhibition Zone Diameter (IZD) and Minimum Inhibitory Concentration (MIC) similar to those of 0.2% Chlorhexidine (CHX) against *Streptococcus mutans*, *Streptococcus salivarius*, and *Streptococcus pyogenes* (5). CHX mouthwash has strong antibacterial activity against different oral microorganisms and is used as the positive control group in many studies. Since *S. mutans* is an acidogenic and aciduric bacterium responsible for caries and also the most important microorganism in bacterial plaque, ANNAS1 can also be used for the prevention of caries and periodontal disease (5). Shafiee et al. showed that the MIC value of CHX for *S. sanguinis* and particularly *Enterococcus faecalis* was significantly higher than that of ANNAS1 (5). The exact mechanism of ANNAS1 mouthwash against bacteria has yet to be understood but it appears to be via the destruction of cell integrity (5). Also, analysis of ANNAS with Nitro Blue Tetrazolium (NBT) test showed that constituents of anbarnesa smoke induce the activity of neutrophils in the tested blood and enhance their antimicrobial activity (12). However, it should be noted that CHX, despite its potent antimicrobial property, has several side effects and shows cytotoxic effects in bactericidal concentrations (15). In another study, Shafiee et al. demonstrated that ANNAS1 mouthwash in 1/64, 1/128 and 1/256 concentrations had antibacterial properties while being nontoxic for L929 fibroblasts and can be used with fewer side effects for plaque control and prevention of periodontal disease and caries (4).

Parvin et al. showed that anbarnesa smoke in direct contact (without solvent) via at least 6 seconds of fumigation created growth inhibition zones on *Pseudomonas aeruginosa* and *Staphylococcus aureus* cultures (11). However, further studies with a more accurate methodology are required to further elucidate this finding. The antibacterial efficacy of anbarnesa is believed to be due to the presence of some antibacterial agents in donkey's dung produced through the process of fermentation and digestion of foods in the digestive system as well as the presence of probiotics (10, 11). Also, it seems that the smoke from burning female donkey's dung increases the permeability of cells for its effective agents (10, 11). Sadeghi Aliabadi

et al. showed the cytotoxic effects of anbarnesa smoke combined with water and n-hexane solvent on HeLa and KB cancer cells. They reported that this solution was non-toxic for L929 fibroblasts in dilutes with higher than 2 mg/mL concentrations (10). Also, based on previous studies, they stated that hydrolysis of dung lignin produces some compounds with bacterial growth inhibition properties (10, 11, 16). Based on their findings, further investigations are required to identify the bactericidal and bacteriostatic compounds in anbarnesa smoke.

In another study, researchers applied ANNAS1 solution on wounds created on the back of the neck of Wistar rats and showed that number of myofibroblasts (which are effective in scar formation) decreased by 3 folds following the application of this solution to superficial and deep areas of wounds after 14 and 21 days, and skin, hair follicles, and sebaceous glands appeared after 21 days in the area as well. This indicates that ANNAS1 solution enhances wound healing and decreases scar formation (3). The process of scar formation is complex and depends not only on the number of myofibroblasts but also on the environment in which their function is regulated. It appears that the effects of ANNAS1 solution on decreasing scar formation relate to regulation of the level of expression of TGF-B1 because scar formation decreases by inhibiting the activity of TGF-B1 (3, 17). However, the exact mechanism of function of ANNAS1 has yet to be clearly understood (3) and further investigations as well as clinical trials are required for its use in medicine and dentistry.

Footnote

Authors' Contribution: Study concept and design: Hassan Ali Shafiee and Elham Moravej-Salehi; drafting of the manuscript: Elham Moravej-Salehi; critical revision of the manuscript for important intellectual content: Elham Moravej-Salehi; study supervision: Hassan Ali Shafiee and Elham Moravej-Salehi.

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