

A Drug-In-Adhesive Transdermal Patch Containing Smoke Of Anbar Nesara: Formulation And Evaluation Of Physicochemical Properties

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Abstract

Background and Aim: Smoke of Anbar Nesara (AN) is one of the types of smoke with wound healing properties. Many dosage forms can be considered for ease of use in patients, including transdermal patches. This study aims to prepare and evaluate physicochemical properties of transdermal patch contains smoke of Anbar Nesara (TP@AN).

Materials and Methods: In this study, smoke from burning AN was collected in propylene glycol and study by Gas chromatography–mass spectrometry method. AN smoke was used to make the TP@AN. TP@AN had a 5% concentration of AN was made using the casting procedure. TP@AN was characterized by some pharmaceutical tests as appearance, swelling, determination of surface pH, adhesion strength, disintegration time and AN release.

Results: The results were showed that 21 compounds were identified in the AN smoke. However, the results of the present study showed that the TP@AN had suitable characteristic in weight and volumetric swelling, surface pH and adhesion strength. The results of AN smoke release from TP@AN after 24 hours was close to 97%,

Conclusion: The results of the present study showed that patches containing smoke of AN have acceptable physicochemical properties. With further research, these transdermal patches can be used in the pharmaceutical industry. And also, be examined based on the dose used to treat various diseases.

Keywords: Transdermal patch, Smoke, Anbar Nesara, Formulation, pharmaceutical tests

1. Introduction

Drug delivery systems have led to tremendous expansion and progress in the field of Pharmacology. In Pharmacology, one of the most vital needs is to find and build suitable drug carriers for drug delivery. As a result, the side effects of drugs are reduced due to the use of small amounts of drugs and efficacy in certain areas. One of the goals of the new drug delivery systems is to increase the safety and adequacy of the drug molecule with the appropriate formulation. Which leads to better patient cooperation in the use of prescription drug (1,2). The transdermal drug delivery system (TDDS) is a non-invasive and efficient method that provides slow release of the drug and delivers the drug to the target site. This system can improve the therapeutic efficacy and safety of drugs, keep the plasma level of the drug constant and is a convenient and painless method. In this type of drug delivery system, they are known as separate and self-closing dosage forms. When placed on healthy skin, it can quickly enter the bloodstream and show its therapeutic effects. Therefore, transdermal drug administration is one of the most important and potential ways to deliver drug locally and systemically (3-8). For the drug to have a therapeutic role, it must be protected until it reaches the target site in the body and retain their chemical and biological properties. Some drugs are highly toxic and can cause negative side effects and if they are destroyed during release, their therapeutic effect is reduced. Since the introduction skin patches until today, only a limited number of drugs are available in patch form, and not much has changed in their structure one of the main reasons, is the limited number of factories that produce patches. Also, drugs that have suitable physicochemical properties for cutaneous drug delivery are limited (9-11).

Natural substances utilized in traditional medicine include the excrement and urine of animals such as elephants, camels, cows, and donkeys (12). Animal excrement products have been studied by researchers from various aspects. Some studies on the feces or waste of various animals have shown that the number of volatile solids in donkey feces was higher than in goats and cows. While the gas produced from goat waste is more than cattle and donkeys(13). Some other studies have shown that parts of the waste have significant oxidative activity, which is mainly attributed to the amount of intermediate metals in them (14). Various methods of evaluating and determining the number of glucocorticoid compounds and their related metabolites have also been performed on horse dung. Perhaps some of the effective compounds and medicinal effects of AN can also be related to these compounds. The nature of the feces and its characteristics vary according to the animal and the quality and quantity of its nutrition (15). In traditional medicine, these natural ingredients are used by burning them and inhaling their smoke (12). Donkey dung smoke, also known as AN, is one of these gases. Female donkey excrement and its burning are utilized medicinally in the indigenous cultures of people in many parts of Iran. AN smoke is the term for this type of consumption, which usually takes the form of indoor fumigation and it has an important place in ancient Iranian culture (16,17). As traditional medicine, the smoke from this natural animal compound is mainly used in the treatment of some viral and microbial diseases, allergic diseases, relief of cold symptoms and treatment of some benign tumors and cysts, especially cysts in the female genitalia (16). AN smoke is advised in the treatment of wounds in traditional medicine, and its antioxidant, antibacterial, antifungal, anti-inflammatory, and wound-

healing effects have been examined by some research (16, 18-20). Some of these effects have been mentioned in the sources of ancient medicine and some of them have been mentioned only in folk medicine in different parts of Iran and have therapeutic applications. Since the use of this natural substance in the form of smoke is challenging in today's world, it is necessary to prepare this substance as a suitable dosage form of medicine and facilitate the use of medication in the patient. Then, AN smoke is used in the repair of genital wounds, including episiotomy in Iranian culture, so the preparation of a transdermal patch of this smoke is a good option to heal wounds in the genital areas. This study aims to prepare and evaluate pharmaceutical properties of transdermal patch contains smoke of AN.

2. Materials and Methods

2.1. Materials

Hydroxypropyl Methylcellulose (HPMC) K100, triethyl citrate and propylene glycol were purchased from Merck (Darmstadt, Germany). Commercial Eudragit® RL100 was obtained from Evonik industries (Essen, Rhine–Westphalia, Germany). Moreover, AN were collected from Shahrekord, Chaharmahalva Bakhtiary province, Iran.

2.2. Preparation of AN smoke

The smoke from AN, based on the device designed in other studies, was prepared with some changes (figure 1). 100 gr of AN was crushed and put inside a balloon. At each stage of burning, sample was heated to a temperature of 300 ° C. Oxygen flow is non-continuously (1 puff every 5 minutes) in this system. Formed smoke was directed to a container of 5 g propylene glycol which was set on stirrer and surrounded by ice. The resulting smoke entered the container and it was continuously mixed with propylene glycol. After burning one kilogram of AN repeatedly and collecting its smoke in propylene glycol solvent. The resulting solution was weighed and diluted to % 83.3 w/w (g of AN smoke/g of propylene glycol) for use in the next step.

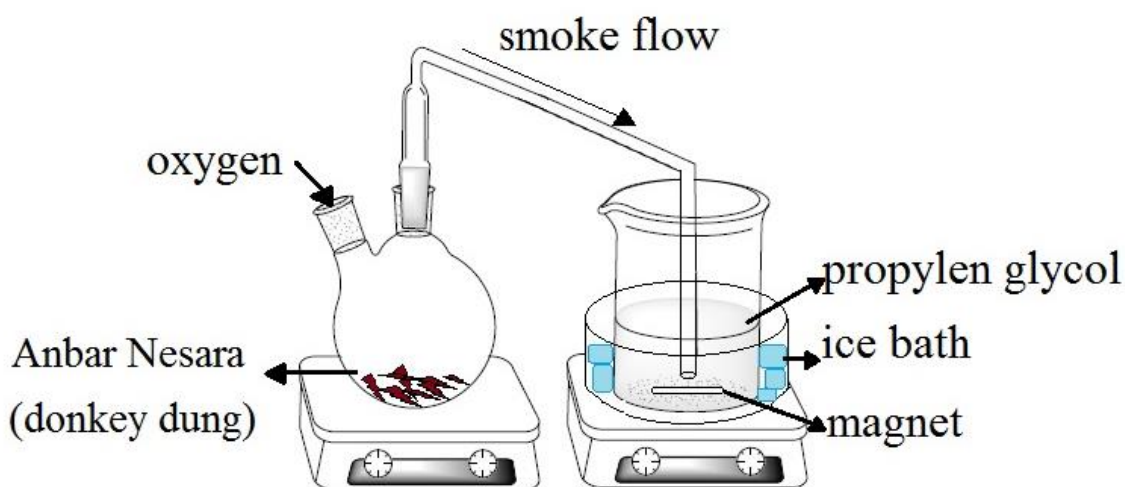


Figure 1. Ischematic of produce and collect Anbar Nesara (A.N) smoke

2.3. Determination of chemical composition of AN smoke

Gas chromatography (GC) and Gas chromatography–mass spectrometry (GC-MS) devices were used to identify the constituents of the smoke. Identifying spectrums, was performed by calculating the Retention index(RI). And with values in different sources was published compared. Also, Mass spectra, was performed to identify the compounds. And identifications made, with using the mass spectra of standard combinations, and use the information in libraries was confirmed. The relative percentage of each of the smoke constituents was obtained according to the area under its curve in the chromatogram spectrum.

2.4. Specifications of the devices used

For gas chromatography, from the GC device, model HP-5973, equipped with FID (Flame Ionization Detector) and HP-5MS capillary column, with a column length of 60 m and an inner diameter of 0.320 mm id, in which the thickness of the stationary phase layer was 0.50 μm , was used. The thermal programming of the column, started from 60 ° C, and after a 10-minute stop, at the same temperature, gradually increased at a speed of 6 degrees per minute, reaching a temperature of 280 ° C. Also, for the GC / MS spectrum, from a chromatograph device connected to a mass spectrometer with the mentioned specifications was used. The temperature of the detector and injection chamber was 250 ° C, and carrier gas (Helium) with a purity of 99.999% was used. The velocity of the carrier gas flow was 1 ml / min.

2.5. Preparation of transdermal patch with AN smoke

In this step, 2400 mg HPMC K100, 250 mg AN smoke (soluble in 300 mg propylene glycol) and 250 mg triethyl citrate were dissolved in 5 ml distilled water. In a separate bowl, 1800 mg of EudragitRL100 was dissolved in 10 ml of acetone. Those two solutions were then mixed together and poured into a 7 cm diameter petri dish to dry at room temperature overnight. An inverted funnel was placed on the plate for controlled manner of evaporating the solvent. After, the dried patches are removed and checked for defects or bubbles. Prepared transdermal patch with AN smoke as TP@AN, are then cut into 2 x 5 cm strips and wrapped in aluminum foil and stored in a glass container at room temperature for the next steps (19). The smoke less transdermal patch was prepared as the same process and material composition except AN smoke (TP@). (Table 1)

Table 1. Composition of transdermal patch with 5% AN smoke formulation

No.	Ingredients	Amounts in formulation	Percent in formulation
1	HPMC K100	2400 mg	48
2	Triethyl citrate	250 mg	5
3	Propylene glycol	300 mg	6

4	AN smoke	250 mg	5
5	Eudragit®RL100	1800 mg	36

2.6. Characterization

2.6.1. Appearance characteristics

The prepared patch was visually inspected to confirm that if the prepared patch had a uniform, soft and bubble-free surface to perform another pharmaceutical analyzes on it.

2.6.2. Surface pH

The patch samples as TP@AN and TP@ were placed in 5 ml of phosphate-buffer at (pH 7.4). Then, the pH at intervals of 2, 4 and 6 hours, by digital pH meter (Metrohm 827, Switzerland) was measured by placing an electrode on the patch surface. PH meter was calibrated with standard buffers before measurement and each time the measuring was repeated 3 times and the mean was calculated.

2.6.3. Determining the weight and volumetric swelling

To measure the patch weight swelling ability, the weighted patch sample was placed on a plate with 50 ml of phosphate buffer pH 7.4 and incubated at 32 ° C. The swollen patch was then weighed at regular intervals to reach its maximum weight and its weight was fixed. The patch weight swelling was calculated by the equation 1.

On other hand, the thickness of the patch from three dimensions were measured by a caliper and swollen patch was investigated from these dimensions again. The patch volumetric swelling was calculated by the equation 2. All the data were repeated 3 times at and their average was reported.

$$\text{Equation 1: \% weight swelling} = \frac{\text{weight of swollen patch} - \text{initial weight of patch}}{\text{initial weight of patch}} * 100$$

$$\text{Equation 2: \% volumetric swelling} = \frac{\text{volume of swollen patch} - \text{initial volume of patch}}{\text{initial volume of patch}} * 100$$

2.6.4. Measurement of adhesion strength

This test was done to calculate the minimum adhesion force, created between the patch and skin. First, piece of cellulose membrane, which was cut to dimensions of 1 × 2 cm², was glued to the behind of a glass container and it was hydrated with distilled water as figure 2. After, one of prepared patch (TP@AN or TP@) which was attached to the behind plate of a balance, was pressed against the surface of the cellulose membrane for one min. Then, water was poured into another plate of balance drop by drop with 3 ml/min speed. The time when the patch was removed from the surface of the cellulose membrane was recorded for calculate the adhesion strength. This experiment was repeated three times, and its average number, was reported.

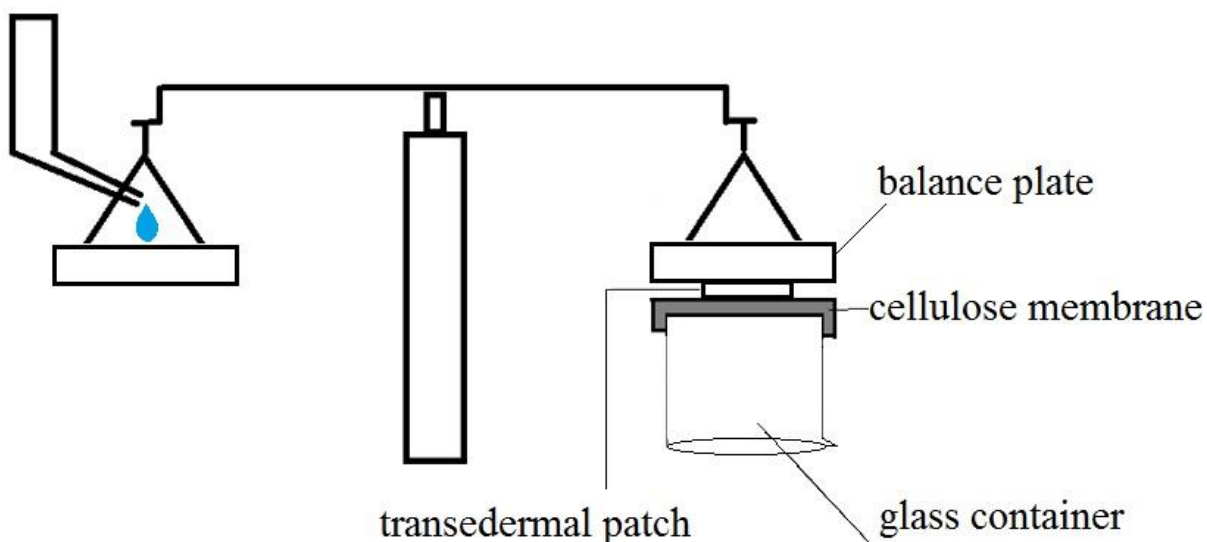


Figure 2. Ischemic of adhesion strength determination method from transdermal patch

2.6.5. Release test

After investigating the obtained smoke sample and its standard curve was determined by light spectrum. Release studies are performed by a permeation cell Franz with a receiver chamber of 20 ml capacity. The cellulose acetate membrane (pore size 0.45 μm) was set between the donor and receiver part. The patch was placed on cellulose acetate membrane and covered with aluminum paper. The receiver chamber of Franz cell was filled with phosphate buffer pH 7.4. The whole assembly was positioned on a hot magnetic stirrer and the solution in the receiver chamber was stirred steadily using a magnet and its temperature was maintained at 32 ± 0.5 °C. Samples were taken at predetermined time and equal volume of phosphate buffer at each sampling time was replaced. Taken samples were analyzed by spectrophotometry to determine the smoke content by obtained standard curve.

3. Results

3.1. Chemical composition of AN smoke

The study of smoke compounds showed that 21 compounds were identified in the AN smoke, which are shown in table 2. The most ingredients were phenolic compounds. The prominent peak belongs to 1-Butanone, 1-(2,4,6-trihydroxy-3-methylphenyl) 1,2-Benzenedicarboxylic acid, diisooctyl ester and Phenol, 2,6-dimethoxy with 12.778 and 12.191 %. In identical research, an important peak of AN smoke belonged to toluene in a mixture with tropylium ion, and another eminent peak was dimethyl benzene (xylene). In their study, the molecular weight of separated compounds was lower than 100 atomic mass units (AMU).

Table 2. Identified Compounds in AN smoke by GC/MS analysis.

Number	Compound	Total %	Retention
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			time (min)
1	o-n-Butylhydroxylamine	6.62%	12.21
2	Tetrahydrofuran, 2-isobutenyl-4-vinyl	6.2%	13.41
3	Cyclopropyl carbinol 3-Pyridinol	11.29%	12.62
4	2-Methoxy-4-vinylphenol	6.03%	15.71
5	Geranyl isovalerate	7.17%	18.31
6	Trimethoxyamphetamine, 2,3,5	16.3%	17.23
7	Cyclohexanol, 2,3-dimethyl Cyclohexanone, 2-(hydroxymethyl)	3.120%	13.70
8	1H-Pyrazole-5-carboxaldehyde, 1-(tetrahydro-2H-pyran-2-yl)	4.02%	16.42
9	1,2-Benzenediol, 3-methoxy	3.012%	14.78
10	2(1H)-Naphthalenone, octahydro-1,1,4a-trimethyl	12.3%	16.64
11	Dimethyl sulfone	6.021%	12.31
12	1-Butanone, 1-(2,4,6-trihydroxy-3-methylphenyl) 1,2-Benzenedicarboxylic acid, diisooctyl ester	12.778%	29.18
13	Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)	4.162	21.45
14	Desaspidinol	6.440	19.83
15	Phenol,2,6-dimethoxy-4-(2-propenyl)	4.156	19.83
16	2-Propanone, 1-(4-hydroxy-3-methoxyphenyl) (+)-s-2-Phenethanamine, 1-methyl-N-vanilly	7.451	21.22
17	5-tert-Butylpyrogallol Trimethoxyamphetamine, 2,3,5	3.123	17.77
18	Cyclohexene, 2-ethenyl-1,3,3-trimethy	3.728	15.46
19	Phenol, 2,6-dimethoxy	12.191	15.88
20	Phenol, 4-methoxy-3-(methoxymethyl) 2,5-Dimethoxybenzyl alcohol	2.88	16.161

21	1,4-Dimethoxy-2,3-dimethylbenzene Ethanone, 1-(4-hydroxy-3-methoxyphenyl)	4.94	18.73
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3.2. Surface pH of transdermal patch

The results of pH determination show that all types of prepared patch have a pH within the normal they don't have any side effects based on tissue irritation (table 3).

Table 3. Surface pH of transdermal patch(n=3).

pH (mv) Patch type	2 hours	4 hours	6 hours
TP@AN	6.34±0.21	6.45±0.13	6.72±0.11
TP@	6.11±0.2	6.21±0.17	6.35±0.19

3.3. Determining the weight and volumetric swelling

Study of weight and volumetric swelling of patch by mentioned method was done and the results were reported in tables 4 and 5.

Table 4. Evaluation of weight swelling of transdermal patch(n=3).

Weight (mg) Patch type	Initial weight (mg)	Final weight (mg)	Weight Swelling (%)
TP@AN	0.0794	0.1355±17.2	70.6±17.2
TP@	0.0166	0.1408±22.1	748.1±22.1

Table 5. Evaluation of volumetric swelling of transdermal patch(n=3).

length (cm) Patch type	Initial length (cm)	Initial width (cm)	Initial thickness (cm)	Final length (cm)	Final width (cm)	Final thickness (cm)	Volumetric swelling (%)
TP@AN	1	1	0.02	1	1	0.08±12.2	300.0±12.2
TP@	1	1	0.01	1	1	0.04±15.3	300.0±15.3

3.4. Adhesion strength

The adhesion strength of the prepared transdermal patches was performed according to the mentioned method, the results of which are shown in (Table 5).

Table 6. The adhesion strength of transdermal patch(n=3).

Patch type	Time (s)
TP@AN	1294.33±131.77
TP@	1121.33±85.76

3.5. Evaluation of in vitro release

Created graph from AN smoke was showed in figure 3. The absorbance at 343 nm versus AN smoke concentration of 3-20 mg/ml were applied. As the results, the obtained equation from calibration curve was $y = 0.108C - 0.3326$ (where y was the absorbance of 1 cm layer, 0.3326 was the slope, 0.108 was the intercept and C is the concentration of the measured solution in mg/ml). Regression analysis reveals a good correlation ($R^2 = 0.9972$). According to figure 4 the AN smoke released from the transdermal patch in the first 1 hour was 55% which represents the initial explosive dose released from the patch. AN smoke release was examined up to 24 hours, which was close to 97%, which was the complete release of smoke after this period.

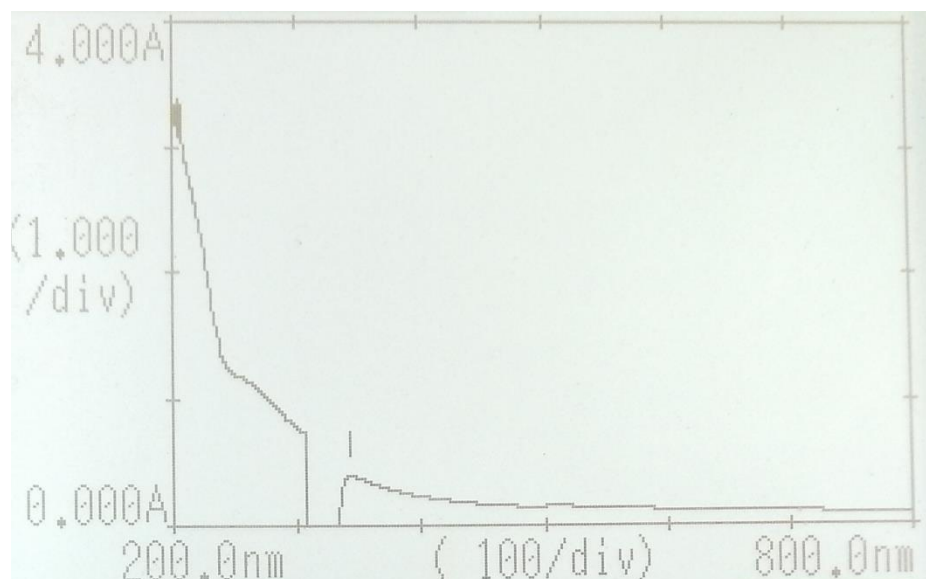


Figure 3. light spectrum of AN smoke by spectrophotometer

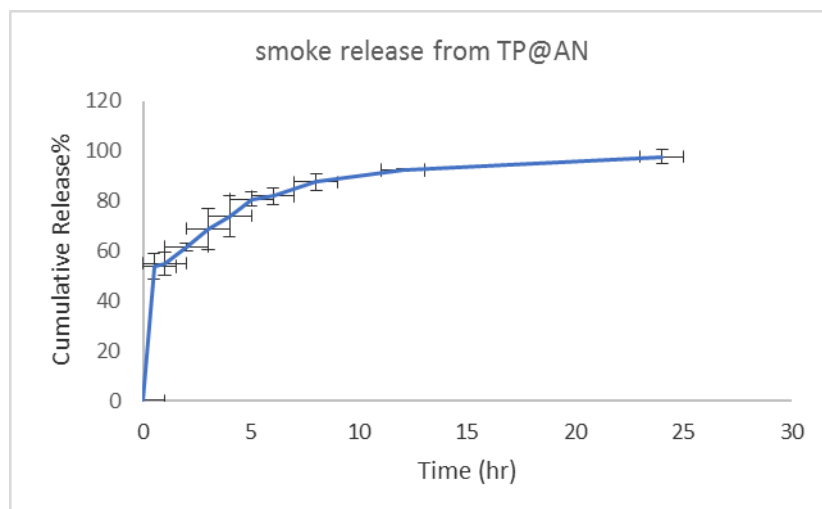


Figure 4. AN smoke release from TP@AN at 32°C and pH=7.4 (n=3).

Discussion

The aim of this study was to prepare and evaluate the properties of transdermal patch pharmaceuticals containing AN smoke. The results of the present study showed that the prepared transdermal preparation patch had acceptable characteristics of appearance, disintegration time, thickness and swelling, surface pH, film adhesion and in vitro release compared to the smokeless patch. Results indicate that this patch can be used in the next steps to be evaluated and used in the treatment of various skin lesions such as skin wounds. Today, skin patches are widely used for drug delivery. Skin patches have been used for various purposes such as drug delivery to reduce pain, heal wounds and treatment of aphthous, control nausea and so on. Dermal drug delivery for the treatment of local or systemic complications has a long history. In ancient Egypt, vegetable oils (castor oil, olive and sesame) and animal fats were made into ointments and creams and were used in the treatment of local diseases (21-25). In a study by Patel et al. (2009) on a skin patch containing curcumin compound, HPMC K4M and Ethyl cellulose. These compounds in different concentrations were used to prepare the skin patch by solvent evaporation method. Oleic acid combination with different concentrations was also used for penetration enhancer for skin permeation. In this study, all formulated transdermal patches were examined for different characteristics. Factors examined in the prepared formulas included drug content, thickness and weight change, folding strength and tensile strength. Based on the results of this study, it was observed that among the prepared formulations, patch prepared with Ethyl cellulose with oleic acid has the highest skin penetration. It was also found to have a very strong anti-inflammatory effect, similar to the standard diclofenac film, in carrageenan-induced edema mice (26). In another study by Vidiolakshmi et al. (2004), transdermal films were used in different proportions using chitosan and polyvinyl alcohol. In this study, Curcumin was used as a model drug. Ethanol, glacial acetic acid and distilled water were used as solvents for curcumin, chitosan and polyvinyl alcohol, respectively. Glycerin was also used as an emollient. The researchers found that the highest release rate of curcumin was from polyvinyl alcohol films, with a decreasing chitosan ratio in the film composition (27). In another study conducted by Martins et al (2018), on the therapeutic effects of films loaded with resveratrol, the results

showed that these films have significant effects on pain as well as reducing the healing time of mouth sores and ulcers compared to the placebo group. Therapeutic effects of resveratrol, in addition to wound healing, were also attributed to the topical drug delivery system using films as a new method in the treatment of oral aphthous and lesions (28). Estradiol skin patch was another type of patch used in the pharmaceutical industry. The topical form of estrogen is used to treat amenorrhoea in women (29). Estradiol was first used as a hydroalcoholic gel to control the symptoms of menopause (30). Nowadays, skin patches have been able to find their place among other forms of medicine and as one of the successful methods in drug delivery and sometimes they are marketed as the only available form.

In traditional medicine, cases of using AN smoke to heal wounds have been mentioned. An article by Ibn Sina mentions that some natural compounds that stop bleeding are papyrus plant, poppy seeds, iron rust, burnt horse and mare dung, burnt donkey dung. Of course, donkey dung, horse and mare dung have the same effect if they are unburned (16).

Animal feces contain large amounts of organic ingredients, nitrogen and large amounts of plant material eaten by the animal (19). It is important to note that the concentration of toxins in the feces is affected by the materials consumed and the digestion of food. In fact, feces can be a rich source of antibiotics. On the other hand, animals have different microflora and the substances in the feces can produce different antibiotics that are effective on different organisms (20).

Studies have shown that the main components of smoke from incineration of sawdust include formic acid, acetic acid, butyric acid, caprylic acid, vanillic acid, syringic acid, dimethoxyphenol, glyoxal, furfural, methanol, ethanol, octanal, acetaldehyde. And some research was said smoke have more than 200 components. Many smoke compounds such as formaldehyde, acetanilide, methanol and some aromatic compounds such as phenols and cresol have bactericidal or bacteriostatic properties. The antibiotic activity of smoke is related to its formaldehyde content. The effect of each of the above compounds is known, but experiments have shown that the effect of a set of smoke compounds is greater than each one. On the other hand, many compounds in smoke and their antimicrobial effects have not yet been identified (19).

These properties are related to smoke from burning wood, while very few studies have been done on dung smoke, its compounds and healing properties, but these effects can be attributed to the composition of food and plants consumed by animals and thus the properties of smoke from dung is justifiable. One of the proven compounds in dung is lignin. Hydrolysis of lignin causes the formation of some compounds with the property of inhibiting the growth of microorganisms. Based on the origin, three main groups of weak acids, eruption compounds and phenolic compounds are formed by this hydrolysis, and the antimicrobial effects of smoke from burning dung are probably more related to one of these three groups of substances (17).

In various studies, the antimicrobial effects of dung smoke have been proven. For example, in a study by Parvin et al., the effect of AN smoke on the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* was investigated. The results were showed that AN smoke can significantly inhibit the growth of these two bacteria. This effect was attributed to the presence of plant compounds in the dung and the resulting smoke (20). Some studies have shown that the smoke from burning pine plants and

animal substances contains monosaccharide derivatives, methoxyphenols, diterpenoids, phytosterols, sterols and chitin derivatives. Therefore, the presence of these substances can justify the antimicrobial effects of smoke (17). Since one of the major problems in wounds is infection with various bacteria, especially *Staphylococcus aureus*, the use of patches containing AN smoke can be an effective step in reducing wound infections.

Regarding the effectiveness of AN smoke in wound treatment, we can mention the study of Safarpour et al. The effects of Guajol® ointment synthesized from the condensate of AN smoke on burn wound healing in rats were investigated. The group treated with 5% Guajol® ointment showed higher levels of collagen fiber and fibroblast cells on the seventh day. The wound of the group treated with 5% Guajol® ointment on day 21 was covered by healthy epithelial and epidermal tissues and hair follicles (18). In this study, the antimicrobial effects of AN against wound infectious agents such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* are well noted.

In the present study, natural animal compounds were used. Due to the side effects of chemical drugs, the use of other natural compounds, including plant and animal, can be considered as one of the low-risk compounds for the preparation of drugs in the form of skin patches. Based on the results performed on the skin patch formulation containing AN and its positive results, further studies on this material can be performed to provide more complementary results in the future.

Conclusion

The results of the present study showed that patches containing smoke of AN have acceptable physicochemical properties. Due to the healing properties of AN smoke can be used in the treatment and improvement of skin-related diseases, especially wounds. This requires further investigation.

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