Opium Carcinogenicity: A Systematic Review of Experimental Studies

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ABSTRACT

Several epidemiological studies have reported that regular use of opium can be associated with an increased risk of developing cancers, including oesophageal, laryngeal, bladder, lung, and gastric cancer. In this systematic review, we aimed at investigating whether experimental studies support this finding and, if yes, how opium consumption can cause cancer. Most of the articles that have studied opium or its derivatives have found it as a carcinogen. However, due to the complex composition, different forms, and various ways of opium use, further comprehensive experimental studies are required. Using modern genomic and epigenomic methods seems to help determine the molecular mechanisms underlying opium carcinogenicity.

Keywords: Opium, Carcinogenicity, Guideline, Neoplasm
INTRODUCTION:

An increasing number of epidemiological studies, especially in Iran, have suggested that opium use could cause cancer in humans. However, there is contradictory evidence about the carcinogenicity of other opioids (1-5). Opium is a highly addictive drug, which could lead to drug dependence and disorder. Opium is the most commonly consumed drug among Iranians, although the opiate trade has been banned since 1997 (6). The most recent national survey indicated that more than 2% of Iranians suffer from opium abuse disorder (7). Opium is a brown, bitter, and dried latex obtained from the unripe seed of Papaver somniferum (1). Over 50 various alkaloids such as noscapine, morphine, and thebaine are derived from the opium poppy. Some of these substances are classified as medicines. Morphine is the main alkaloid of opium. The mode of action of morphine and its derivatives relies on these alkaloids’ action as mu and kappa opioid receptor agonists that later derive into analgesia. Large amounts of morphine are prescribed for moderate to severe pain in cancer patients each year (8, 9). Noscapine is another important alkaloid derived from opium used in medicine and acting as a sigma receptor agonist (10). Unlike other opioids, noscapine does not cause addiction. Some studies indicate that this opium alkaloid can demonstrate anti-carcinogenic properties (11-16). There are numbers of traditional narcotic derivatives of opium in the list of illegal narcotic drugs, including Teriak (air-dried and dark, sticky, or crumbly paste of raw opium), Shireh (refined opium made by boiling the raw opium or Teriak in hot water, and heating and passing it through filters for several times), Sukhte (dry residue of the burned Teriak), and Tofaleh (residue of the filtered Teriak solution) (3, 8). Recent evidence suggests that the rate of opioid consumption is increasing in 25 OECD (Organisation for Economic Co-operation and Development) countries (17). Although many studies on humans have shown a higher risk of cancer incidence among opium users, opening the black box of the molecular pathways and mechanism of opium carcinogenicity is a challenge for health researchers. For instance, it is unclear whether opium is a genotoxic carcinogen or a non-genotoxic carcinogen (18-21). This study attempted to systematically review experimental studies, including in-vivo and in-vitro, to explore opium carcinogenicity.

METHODS

Search strategy and selection criteria:
We searched PubMed, Google Scholar, and Scopus to identify experimental studies on opium use and cancer. We searched PubMed with the terms “Opium”, “Neoplasms” [MeSH term], “Carcinogenesis” [MeSH term], “Animal” OR “In-vitro”, “Cell Line” OR “In-vivo”, and “Experimental Study”. Entry terms were used to search Google Scholar and Scopus databases. The PubMed search was limited to “Other Animals” (for species), and the Scopus search was filtered by “Article” (for document type), “Medicine” (for the subject area), and “Non-human Subjects” (for the keywords). All searches were updated in September 2019. No language limitation was applied. However, all found publications were in English.

Data extraction:
A total of 3067 articles were found through crude searches. After removing duplicates, 2016 unique records were screened. Of these, 1926 studies were excluded at the title and abstract evaluation phase for several reasons according to the exclusion criteria mentioned above. Finally, 90 articles were screened for full-text (Figure 1).

RESULTS:
Among 90 full-text screened articles, 36 were about opium or its alkaloids, which were carefully studied,
and the data table was prepared. Data were extracted based on the bibliography (first author, year, type of opium sample, and the type of study) and results of the selected articles. The summary of the results obtained from these 36 articles, including 52 tests, is presented in Table 1. The complete table of opium derivatives and opium alkaloids (noscapine, morphine, and heroin) are provided in Table 2 and Supplementary Table 3.

**Noscapine**

Thirty-five studies (23 articles) were on noscapine alkaloids. Among these, 22 were in-vitro studies, and 21 experimental studies reported that noscapine could inhibit cancer growth (15, 16, 22-39). One article did not report any effects (40). There were 13 in-vivo studies among noscapine articles. Interestingly, all of them indicated the cancer-protective effect of noscapine.

**Morphine and Heroin**

Four articles investigated the effects of morphine and heroin on cancer. Among these, two studies indicated the tumor-suppressive effect of morphine, while one reported its carcinogenicity (4, 41, 42). One article also reported that heroin decelerated tumor growth in mice (43).

**Opium**

Nine articles examined the carcinogenicity of opium or its common derivatives like Teriak, Shireh, Sukhteh, and Tofaleh. Some of them included both laboratory tests and animal models, whereby the results of the in-vivo and in-vitro studies were separately reported in Table 2. Most of these articles indicated the carcinogenicity of opium or its derivatives. We re-evaluated their data and matched the similarity among materials.
and methods with standard carcinogenicity criteria, including OECD and ARRIVE (Animal Research: Reporting of In-Vivo Experiments) guidelines, standard test conditions, dose-response association, etc. (51-54) It was finally found that only nine studies had applied carcinogenicity tests on opium. Among these studies, 13 tests were performed on cancer cells or laboratory animals. Data on these nine articles are presented in Table 2.

Tables 3 and Table 4 summarize the results of the re-evaluation of articles in terms of testing raw opium and matching them with standard guidelines (51-54). The quality (high, moderate, and low quality) of strains, animals, and cells were determined by matching them with previous guidelines based on using suitable materials; checking strains for contamination, sensitivity, and mutation; proper concentration and condition during the procedure; and considering pre-incubation in the studies.

As an illegally marketed drug, opium contains various types of plant alkaloids as well as impurities. Supplementary Tables 1 and Table 2 provide an example of these materials. These data were obtained from the analysis of a sample of opium and its four well-known derivatives, Teriak, Shireh, Tofaleh, and Sukhteh (the Iranian names of these products), at the Cancer Biology Research Centre (CBRC) of Tehran University of medical sciences.

### Table 1. Summary of the studies results

| Substances | Type of Study | Technique | Result | Comment |
|------------|---------------|-----------|--------|---------|
| Opium (9 articles including: 13 tests) | In vitro (8) | 4 (Bacteria reverse mutation) 2 (Mammalian cell assays) | Carcinogen (6) | Carcinogenicity test |
| Noscapine (23 articles including: 35 tests) | In vitro (22) | Cancerous cell lines | Carcinogen (0) Anticancer effect evaluation |
| Other Opioids (Morphine & Heroine (4 articles) | In vitro (1) | Cancerous cell lines | Protective (1) Anticancer effect evaluation |
| | In vivo (3) | Rat | Carcinogen (1) | Carcinogenicity test |
| | | Mice and Rat | Protective (2) | Carcinogenicity test, Anticancer effect evaluation |
Table 2. In-vivo and in-vitro studies, indicating the carcinogenicity effect of opium and its derivate

**Opium**

| In vitro (bacterial reverse mutation test) studies |
|-----------------------------------------------|
| Author (Year) | Type of opium or extraction | Cell line type/ Animal Species | Concentration | Technique | Clinical Index | Result | Carcinogenicity | Risk of Bias |
|-------------------|-----------------------------|--------------------------------|---------------|-----------|----------------|--------|----------------|-------------|
| 1 Mottaghi M. A. (2018) | Opium | S. typhimurium TA100 | 0.001- 0.01- 0.02- 0.04- 0.08- 0.16 g/ml | • Ames test | • Mutation | Not mutagenesis in 0.001 but mutagenesis in other dosages | carcinogen | 4 |
| 2 Friesen, M. A. (1985) | Opium pyrolysates | S. typhimurium TA98 | • Ames test | • HPLC | mutagenesis | very mutagen | carcinogen | 4 |
| 3 Malaveille, C. (1982) | Opium pyrolysates and sukhteh | S. typhimurium TA98 and TA100 strains | 30-550 mg | • Ames test | • Plate incorporation assays | Induce mutation | carcinogen | 4 |
| 4 Hewer, T. (1978) | Opium pyrolysates | S. typhimurium TA98 and TA100 | 4.16 mg | • Ames test | • Preparation of liver post-mitochondrial fraction | mutagenicity | Induce mutation | carcinogen | 4 |

| In vitro (mammalian cell assays) studies |
|---------------------------------------|
| Author (Year) | Type of opium or extraction | Cell line type/ Animal Species | Concentration | Technique | Clinical Index | Result | Carcinogenicity | Risk of Bias |
|-------------------|-----------------------------|--------------------------------|---------------|-----------|----------------|--------|----------------|-------------|
| 5 Khaleghi, M. A. (2016) | Opium | A431 cell line A.G.S. cell line Hela cell line HepG2 cell line MCF7 cell line N26 cell line PC12 cell line WEHI cell line | 2.86 x 10^{-4} g/ml | • M.T.T. assay used to study cell viability | • Cell Proliferation | Induce apoptosis | protective | 4 |
| 6 Arababadi, M.K. (2015) | Opium | Jurkat cells | 2.86 x 10^{-5} g/ml | • Annexin V staining | • R.N.A. extraction, reverse transcription, and quantitative real-time PCR | Apoptosis | Change in apoptosis rate of the cell line | protective | 4 |
| 7 Friesen, M. A. (1985) | Opium pyrolysates | Syrian hamster embryo cells, C3H, IOT1/2 cells | 0.5 mg/ml | • M.T.T. assay | • Preparation of mitochondrial fraction | Apoptosis | Induce apoptosis | carcinogen | 4 |
| 8 Perry, P. E. (1983) | Opium pyrolysates and sukhteh | Chinese hamster ovary (C.H.O.) cells and Human peripheral blood lymphocytes and Salmoneila typhimurium TA98 | 30 μg/ml | • Silver chromosome exchange (S.C.E.) | • Making s9 mixture | • Preparation of liver post-mitochondrial fraction | Mutations induction | Induce mutation | carcinogen | 4 |
| Author (Year) | Type of opium or extraction | Cell line type/ Animal Species | Number of animal in each group | Dosage/route of exposure/time of exposure | Technique | Clinical index | Statistical test | Result | Carcinogenicity | Risk of Bias Assessment |
|---------------|----------------------------|-------------------------------|-------------------------------|------------------------------------------|-----------|----------------|-----------------|--------|----------------|------------------------|
| 9 (Alzaidi,M.A. 2018) | Opium | Male Wistar rats 140-180 g | 54 rats divided into 3 groups treated with: 1. purified water 2. DEN 3. opium (experimental group) | 300 mg/kg Oral 16 weeks (5 times in a week) | Hematoxylin and eosin stain RT-PCR | Histopathology changes Gene expression | ANOVA | • No carcinogenic changes were observed in the opium-treated animals at the end of week 20 • The treatment of animals with opium significantly inhibited the increased level of CDK2 • Opium did not induce significant alteration in the expression of p53, p21, cdk2, e-Cdh, and n-Cdh genes involved in the gastrointestinal tumors. | Protective | 5 |
| 10 Tsuda,H. 1993 | Opium | male F344 rats | 260 rats divided into 3 groups (2 groups in experiment 1 followed in 3 groups in experiment 2) Experiment 1-2: 1. DEN/IQ/Captopril/HCE/DES-OP/DDT/HCB 2. saline/corn oil/DMSO/corn oil 3. saline/corn oil/DMSO/corn oil (different dose) | 60 mg/kg Intraperitoneal (I.P.) 2 weeks | Glutamine s transferase p form positive liver cell focus | • Number of Glutamin s transferase p form positive | Student’s t test | • Not a promoter but have a carcinogen effect | carcinogen | 5 |
| 11 Friesen,M. 1985 | opium pyrolysates | female Syrian golden hamsters, 8 weeks old | 3 groups of 10 female Syrian golden hamsters: 2 experimental groups 1 control group | 1.659 mg/animal intratracheal instillations 114 weeks (once in a week) | Transformation assays | • Body-weight • Survival rate | Not mentioned | • Hyperplasia • No change in body weight | carcinogen | 5 |
| 12 Friesen,M. 1985 | opium pyrolysates | C57BL/6 mice and female C.B.A. mice 20-24 weeks old | 3 groups of 27 female and 30 male C57BL/6 mice: 2 experimental groups 1 control group | 40 mg O.P. per mouse Oral Subcutaneous injection 114 weeks (once in a week) | Mass spectrometry HPLC UV spectrophotopy H-Fourier transform nuclear magnetic resonance (1 H.FTNMR) spectrometry Preparation of liver post-mitochondrial fraction | • Morphological change | Not mentioned | • Tumorogenesis result | carcinogen | 5 |
| 13 Friesen,M. 1985 | opium pyrolysates | female Swiss mice (SPI) 21-day-old | 30 female Swiss mice | 28.8 mg O.P. Mouse skin test 114 weeks (once in a week) | Tests on mouse skin | • Tumor size | Not mentioned | • No increase | No effect | 5 |
| Study type | Follow the carcinogenicity guidelines | Number or Code of guideline/series | Authors/year | Standard test condition | Strain/cells/animal characteristics | Strain/other animal type | Using activation system (in vitro tests) | Treatment procedures | Standard duration time (in vivo tests) | Carcinogen | Dose-response relationship |
|------------|--------------------------------------|----------------------------------|-------------|------------------------|-----------------------------------|-------------------------|---------------------------------------|----------------------|---------------------------------|--------------|------------------------|
| In vivo    | Yes                                  | Similar to OECD (TG. No.471)    | Mottaghi.M. 44 (2018) | Standard               | High                               | suitable                | Used                                  | High                  | Standard                        | Carcinogen  | Considered              |
|            |                                      |                                  | Friesen,M. 5 (1985)** | Standard               | Moderate                           | suitable                | Used                                  | Moderate              | Lack of enough information      | Carcinogen  | Considered              |
|            |                                      |                                  | Malaveille,C. 45 (1982) | Standard               | High                               | suitable                | Used                                  | Moderate              | Standard                        | Carcinogen  | Considered              |
|            |                                      |                                  | Hewer,T. 46 (1978)     | Standard               | Low                                | suitable                | Used                                  | Low                   | Lack of enough information      | Carcinogen  | Considered              |
|            | Yes                                  | Similar to OECD Series No.214    | Friesen,M. 47 (1985)   | Standard               | Moderate                           | suitable                | Used                                  | High                  | Carcinogen                      | No information |
|            | Yes                                  | Similar to OECD (TG. No.479)**   | Perry,P.E. 49 (1983)   | Standard               | High                               | suitable                | Used                                  | High                  | Standard                        | Carcinogen  | Considered              |
| No †       | Propidium iodide Cell Cycle Staining Protocol | Arababadi,M. 48 (2015) | Standard | Standard | Standard (followed by commercial kits) | Protective |
|            | MTT test protocol and PI staining   | Arjaghil,M. 47 (2016)          | Standard | Standard | Standard (followed by commercial kits) | Protective |
| In vivo    | Yes                                  | Similar to TG. No.451           | Friesen,M. 7 (1985)† | Standard               | Low (less than standard number)   | suitable                | Low (no information about dose selection) | Not standard duration (too short) | Standard                        | Carcinogen  | Not considered          |
|            |                                      |                                  | Alzaidi,M.A. 21 (2018) | Standard               | Moderate (less than standard number) | suitable                | Low (no information about dose selection) | Not standard duration (too short) | Standard                        | Protective |
|            |                                      |                                  | Tsuda,H. 50 (1993)     | Standard               | High                               | suitable                | High                                  | Not standard duration (too short) | Standard                        | Carcinogen  | Considered              |

*The quality of strain, animal and cells determined by level of matching with guideline based on the using suitable material, checking strain about contamination, sensitivity and mutation, suitable concentration and condition during the procedure and considering pre-incubation in the studies (High quality, Moderate quality and low quality).
**Guideline NO.479 was deleted on 2nd April 2014
***The mentioned article includes 3 types of studies
†There is no approved OECD guidelines for this two protocols
††Friesen 1985 article includes 3 animal carcinogenicity studies on opium pyrolysates with different administration route
†††Duration time is around 12 month (carcinogenicity studies normally should be 24 months but 12 month is acceptable too). In this case the number of administration in a week is not acceptable
DISCUSSION and CONCLUSION

Epidemiological studies have reported that regular use of opium can be associated with an increased risk of several types of cancer. In this systematic review, we explored whether the experimental studies could support the carcinogenicity of opium, although the studies on this issue are limited. Among the available studies, nine articles that had performed 13 different cell or animal tests on opium and its derivatives were selected to be assessed. Most of these assays confirmed the carcinogenicity of opium.

Although many articles have worked on opioid alkaloids (mainly noscapine and morphine), they have not considered these materials' carcinogenicity in their investigations. These compounds have been studied on cancer cell lines or animal models (22-40). Nociceptor and morphine have many clinical applications and therapeutic effects. Morphine is used as a potent analgesic drug in treating cancer patients (55). Therefore, it is not expected that such well-known and widely used products, which have undergone numerous efficacy and safety tests, show carcinogenic effects.

Available opium and its derivatives in the black market contain many impurities (See Supplementary Tables 1 and 2), including phytochemical composition, lead, or toxic heavy metals and various substances (some of which are toxic) (66, 57). This means that such substances can play a role in opioid-related harms among people who regularly use opium (58, 59). Therefore, studies on opium alkaloids may not be helpful to show the toxicity of these impurities or the carcinogenicity of crude opium. Further studies are required to address this issue specifically.

Table 4. Result of matching articles with ARRIVE guideline

| Article | Ethical statement | Study design | Experimental procedures* | Experimental animals | Housing and husbandry | Welfare-related assessments | Sample size | Experimental outcomes | Statistical methods | Allocating animals |
|---------|------------------|-------------|--------------------------|----------------------|-----------------------|--------------------------|-------------|----------------------|-------------------|-----------------|
| Alzaidi, M.A. (2018) | approved by the institutional Animal care and use Committee | Considered | Group animals in cage | Not standard procedure | Standard strain | Housing and husbandry conditions: 25±1 °C temperature and 60% humidity under controlled light (12-h light/12-h dark), free access to food and water | acclimatization to the environment for 2 weeks | Less than standard number | Clearly defined | Lack of enough information |
| Friesen, M. (1985) | No information | Lack of enough information | Group animals | Not standard procedure | Standard strain | Housing and husbandry conditions: 23±2 °C temperature and 60% humidity under controlled light (12-h light/12-h dark) | acclimatization to the environment | Less than standard number | Clearly defined | Provided |
| Tsuda, H. (1993) | No information | Lack of enough information | Group animals in cage | Not standard procedure | Standard strain | Housing and husbandry conditions: Housed five per plastic cage on wood chips for bedding | acclimatization to the environment for 1 weeks | Standard | Clearly defined | Provided |

*Important features which are considered as indexes for experimental procedure include: concentration, route of administration, time of day, and duration (explained in details in Table 3)
On the other hand, among the studies aimed at observing or rejecting the carcinogenic effects of opium and its derivatives, most have confirmed these compounds’ carcinogenicity (5, 44-46, 49, 50). Nearly all these studies have been conducted based on known tests or well-known protocols for carcinogenicity (51-54). All studies reported the Ames test result on opium have shown that it is mutagenic (5, 48-50). This result from the Ames test indicates the probability of its carcinogenicity. Four studies have been done on mammalian cells, two of which have reported the carcinogenicity effect of opium (5, 49), while two have reported the protective roles of opium against cancer (47, 48). Of the five animal studies, three showed carcinogenicity of opium (50); one study found no effect (5), and one study showed its protection against tumor progression (20).

We could not find any comprehensive experimental research articles studying the carcinogenicity of opium. Several reasons explaining the lack of such studies are as follows:

Carcinogenicity tests usually require several related and sequential steps to be taken to lead to the necessary results. Completing all the steps is usually complicated and time-consuming. Most of the current guidelines for carcinogenicity tests involve long-term bioassays (several weeks or months) in the animal laboratory. Due to the long period, cost, and need for special facilities and equipment, such studies are beyond many researchers and research centers’ reach. In addition, opium is used by various routes, such as ingestion, smoking, or inhalation. However, there has been no comparison among different routes of use in experimental investigations. Besides, opium smoke contains large amounts of potentially combustible carcinogenic compounds such as Poly Aromatic Hydrocarbons that do not exist in the crude opium itself. Furthermore, opium in the black markets contains numerous impurities that can affect the analyses and make experimental study design complicated. Classic carcinogenicity tests are a set of complementary laboratory and animal tests, while none of the conducted studies have performed or completed all series of tests. Thereafter, the exact pathway of the carcinogenicity of opium is still unclear, and future experimental investigations are required in this regard. Moreover, the use of new genomic and epigenetic screening techniques is not observed in most of the included studies. In conclusion, crude opium has a complex composition and has many impurities that vary in the consumer market. This makes experimental research on this material challenging. There is not much experimental research on this substance. Thus, we could not find any comprehensive experimental research articles studying the carcinogenicity of opium. However, most of the studies found in this search indicate that this substance can be carcinogenic. Through empirical research, further studies are needed to provide an accurate answer to whether opium is carcinogenic and what molecular mechanisms are involved.

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