

# الطريقة المبتكرة للتخلص الآمن من بعض المخدرات المضبوطة

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## الملخص

مع ظهور الحضارة الإنسانية انتشار المخدرات وتعددت مصادرها، ثم **بدأ** انتشرت انتشارا واسعا، بحيث لا تخلو أى بلد منها، مما أصبح خطرها يهدد حضارات الأمم. ولذا فرضت الدول باختلاف جنسياتها قوانين تجرم وتحد من نشاط تجارة المخدرات، مع اتخاذ جميع السبل الممكنة لضبط المواد المخدرة والتخلص منها. وقد أدى ذلك إلى ظهور مشكلات التخلص الآمن من مثل هذه المواد، حيث إن الطرق الشائعة للتخلص منها انحصرت في حرقها إما في الهواء الطلق أو بترميدها، مما يؤثر سلبياً وطبياً على القائمين بالعملية نفسها، أو من يتعرض لدخان الحريق في البيئة المحيطة. وقد دعا ذلك لجنة خبراء العقاقير المخدرة المنبثقة من الصحة العالمية بالأمم المتحدة إلى إصدار توصيات باستخدام طرق بديلة آمنة ورخيصة مثل الطرق البيولوجية أو الكيميائية أو كليهما للتخلص من هذه المواد.

وهدفت هذه الرسالة الى استكشاف طرق آمنة لتحطيم المواد المخدرة مثل الطرق البيولوجية أو الطرق الكيميائية، بديلة لطريقة الحرق المستخدمة حالياً في دول العالم وفي مصر، مع التأكد من أن هذه الطرق لا ينتج عنها مواد لها أي أضرار بيولوجية على الكائنات الحية.

وفي حالة استخدام الطرق البيولوجية، تم عزل سلالات بكتيرية من مادة حشيش متعفنة على بيئة نمو بكتيري. واختبرت قدرة هذه السلالات على تحطيم المادة

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الفعالة المستخلصة من الحشيش والمضافة الى بيئة نمو بكتيري بسيطة خالية من مصدر كربوني غير المادة الفعالة للحشيش. ولكن هذا الاختبار أعطى نتيجة سلبية مع جميع السلالات المعزولة. وقد أدى ذلك الى الاتجاه الى استعمال الطرق الكيميائية لتحطيم مثل هذه المواد المخدرة.

وفي حالة استخدام الطرق الكيميائية، بنى الاتجاه إلى تحطيم المادة الفعالة في المواد المخدرة (سواء أكان حشيشاً أم هيرويناً) على إجراء عملية أكسدة لمجموعات الهيدرو وكسيل في كلتا المادتين، مما ينتج عنه مواد غير فعالة أو إجراء تحليل مائي لمادة الهيروين لينتج منها مواد مفيدة طبيًا. وبناءً على هذا الاتجاه تم استخدام حامض النترريك المخفف ومركب من بيكرومات البوتاسيوم و حامض الكبريتيك المركز (مركب النيكلوكس) ليقوم أي منهما بعملية الأكسدة مع مقارنتهما أيضا ببعض الأحماض والقلويات الأخرى مثل حامض الكبريتيك المخفف و حامض الهيدروكلوريك وهيدروكسيد الصوديوم. وقد نتج عن هذه المحاولات نتائج إيجابية مع مركب النيكلوكس بخلطه مع المادة المخدرة أو حامض النترريك المخفف بتركيز ٥٪ وذلك بغليه مع المادة المخدرة لمدة خمس دقائق.

وقد استخدمت طريقة الفرد الكروماتوجرافي على طبقة رقيقة من السليكا جل (CLT) للكشف إما عن المواد المخدرة أو عن ناتج المعاملات الكيميائية السابق ذكرها. وقد أظهرت نتائج تلك المعاملات الكيميائية اختفاء جميع مركبات الحشيش والهروين والمورفين بالمقارنة بالمركبات القياسية لكل منهم. كما أظهر الكشف عن تلك المواد المتحولة باستخدام طريقة التحليل الغازي الكروماتوجرافي (CG) مركبات مغايرة عن المركبات القياسية للحشيش والهروين والمورفين.

ومن هذه النتائج استخلصنا أن استعمال حامض النترريك المخفف بنسبة ٥ ٪ هو الأفضل من الناحية الاقتصادية في تحطيم المواد المخدرة رغما عن أن استخدام مركب النيكلوكس أعطى أيضا نتيجة إيجابية مقارنة لفعل حامض النترريك، إلا أن استخدامه يعد مكلفا اقتصاديا بمقارنته بتكلفه استخدام حامض النترريك المخفف .

وقد اختبر تأثير المركبات الناتجة من تكسير المواد المخدرة المشار إليها على حيوانات التجارب (ذكور فئران التجارب)، وقد تم حقنها عن طريق الفم إما بجرعة وحيدة

ليوم واحد أو بجرعات متكررة على أربعة أيام، وذلك للتأكد من الآثار الطبية لهذه المركبات على الكائنات الحية. وعموماً أظهرت نتائج تلك التجارب عدم تأثير إنزيمات ميكروزومات الكبد وخاصة إنزيمات الأريل هيدروكربون، والدايميثيل نيتروزأمين-ن-ديميثيلاز، والجلوتاثيون، والجلوتاثيون-س-ترانسفيريز. وهذا يدل على أن المركبات المختبرة ليس لها تأثير سام على خلايا الكبد.

ومن جهة أخرى، أدت المركبات المشار إليها إلى زيادة مستوى السيتوكروم P450 في حالة الجرعات المتكررة، وزيادة مستوى الجلوتاثيون في حالة الجرعة الوحيدة، ولكنها خفضت مستوى السيتوكروم b-5 سواء في حالة الجرعة الوحيدة أو الجرعات المتكررة، وهذا يعد نوعاً من الأنظمة الخلوية الدفاعية التي تمنع عملية التسمم بكثير من المركبات السامة. ومع ذلك لوحظ زيادة طفيفة في مستوى الدايميثيل نيتروزأمين-ن-ديميثيلاز وحامض الثيوبوربيتوريك في حالة الجرعات المتكررة.

وأظهرت النتائج المختلفة لهذه التقديرات أن المركبات الناتجة من تكسير المواد المخدرة نظيرة الدراسة لم يكن لها أي تأثير معنوي على خلايا الكبد أو على الإنزيمات المختبرة في هذه الدراسة.

## **NOVEL METHOD FOR DEGRADATION OF SOME NARCOTICS AND ASSESSMENT OF THEIR TOXIC EFFECTS**

**Dr. Mahmoud. El-Sayed Ali**

### **ABSTRACT**

Degradation of narcotics in Egypt and many other countries is only achieved by burning in open-air furnaces. This primitive method leads to environmental pollution and causes detrimental side effects. Therefore, the aim of the present study is to find safe and clean methods for degrading hashish and heroin. Treatment of hashish or heroin with 5% nitric acid and/or Nicloux reagent 2% potassium dichromate in concentrated sulphuric acid) were used for degradation of these compounds. Thin Layer Chromatography (TLC) and Gas Chromatography (GC) showed that the active ingredients of both hashish and heroin were completely degraded. In order to evaluate the safety of the degraded products, they were injected into the experimental animals (mice) to show the changes in the activity of drug-metabolizing as well as drug-conjugating enzymes. The hepatic content of cytochrome P450 did not change after single dose treatment (24h) on male mice with the extract of degraded hashish and increased after repeated doses by 67%. On the other hand, degraded heroin induced the hepatic content of cytochrome P450 after single- and repeated-dose treatments by 31% and 104% respectively. The activities of aryl hydrocarbon hydroxylase and dimethylnitrosamine-N-demethylase did not change after single and repeated dose-treatments of male mice with degraded hashish and heroin. The idea of the degradation of some narcotics depends on the destruction of narcotics by changing their structures via the oxidation of oxy- groups in these structures.

It is concluded that degradation of hashish or heroin with nitric acid is an economic, safe and clean method.

## 1. INTRODUCTION

Degradation of narcotics in Egypt and many other countries is only achieved by burning in open-air furnaces. This primitive method of destruction leads to environmental pollution affected by the smokes produced during the burning process. These smokes definitely contain the active substances of narcotics. The previous method causes detrimental effects on either the individuals handling the narcotics or others in the surrounding in the course of the smoke<sup>(1-3)</sup>. Narcotics have their own specific clinical picture. They are known to have several toxic effects on human body, with specific action on certain organs. The action of narcotics depends on many factors; one is the route of administration, where the intravenous is the most effective route, followed by inhalation, intra-muscular, subcutaneous then finally through the skin<sup>(4-9)</sup>. The Commission of Narcotic Drugs (CND) at UN discussed regularly the methods that can be used for destructing and seizing narcotic drugs and psychotropic substances. It was agreed that the diversity of the chemical and physical properties of those substances could identify the most suitable method of destruction. As a result of the previous discussion, CND has ended with valuable recommendations emphasizing on: a) the methods of destruction should be cost- effective in relation to other available alternatives, safely conducted and environmentally sound, and should allow for a choice wherever possible; b) during operational field conditions, a due balance must be struck between operator safety , environmental concerns and security; c) in view of the significance of using the biological or chemical treatment as an alternative economical and effective drug-destructive method, research and development in this area should be intensified, and the fund to support and co-ordinate such activities should be made available to the division of Narcotic drugs at the United Nations Secretariat<sup>(1)</sup>.

Therefore, this thesis aimed to find out a safe way to destruct narcotic drugs replacing the direct burning method used in Egypt and other countries, with examining if the degrading materials produced from such alternative method(s) have any biological hazards on experimental animals.

## 2. MATERIALS AND METHODS

### Biological treatment of hashish

One gram of hashish extract was suspended in 100ml citrate-phosphate buffer (C-P) contains per liter (2.45g citric acid, 8.71g  $K_2HPO_4$ , pH 6.8). A serial dilution down to  $10^{-4}$  was made from the original suspension in C-P buffer. 100 $\mu$ l of the last dilution was plated using the spreading technique onto the surface of nutrient agar plates (contains per liter: 3g beef extract, 5g peptone, 5g NaCl, 15g agar, pH 6.8). The plates were incubated at 37°C for 48hrs. Bacterial colonies appeared on the surface of the plates were further purified on nutrient agar medium.

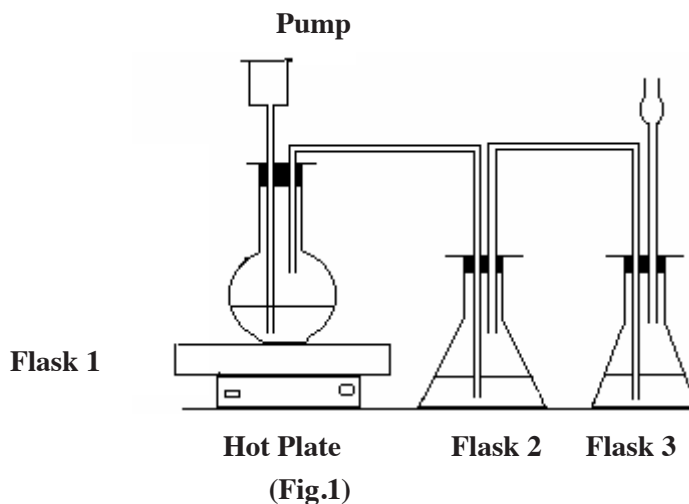
The purified bacterial isolates were tested to utilize the active component of hashish material as the sole carbon source in a minimal medium contains per liter: 12.8g  $K_2HPO_4 \cdot 7H_2O$ , 3.0g  $KH_2PO_4$ , 0.5g NaCl, 1.0g  $NH_4Cl$ , pH 6.8. The medium was supplemented with 1% of the hashish active component extracted by 100% ethyl alcohol<sup>(10)</sup>.

### Chemical treatment for clean degradation of narcotics

#### 2.1. Apparatus used for degrading hashish and heroin

An apparatus (Fig. 1) for chemical degradation was designed to obtain the complete destruction of the narcotics (hashish and heroin). The apparatus consists of three conical flasks (500 ml) connected with glass tubes as shown in Figure (1). Conical flask (1) contains the substance (hashish or heroin) and the destructive reagent while conical flask (2) is used as the first trap step contains cold distilled water to dissolve the gas evolved ( $NO$ ,  $NO_2$ ) during the boiling of the mixture. Conical flask (3) is used as a second trap step contains petroleum ether (use only with hashish) to dissolve hashish vapor which probably can be evaporated during the process.

Several sets of experiments were carried to determine the optimum time and concentration of reagents suitable for degrading the narcotic.



## 2.2 . Procedure:

Sample of hashish (0.5) or heroin (100 mg) were put to flask (1) (Fig. 1), then 50 ml of nitric acid were added (different concentrations were used to determine the optimum concentration required for complete destruction of the narcotics). Flasks (1), (2), (3) were connected as shown in Fig. (1). The mixture was boiled for a fixed time (different times of process were used to determine the minimum time can cause complete destruction of narcotics). Samples of the boiling mixture were taken, allowed to cool at room temperature, and then extracted with chloroform for heroin or with petroleum ether for hashish samples. The extract was analyzed by TLC and by GC techniques as described in section (2.3, 2.4 ). Water in flask (2) was taken to detect nitric acid which may be formed by dissolving NO, NO<sub>2</sub> in water during the boiling of nitric acid reagent with the narcotics. Sample of petroleum ether in flask (3) was taken to detect hashish vapor which probably can be evaporated during the process.

## 2.3 Detection narcotic material by Thin layer chromatography (TLC)

A mixture of raw material and liquid contains nitric acid(10 g) was added to 15 ml of petroleum ether to extract the active components of hashish or its destructive component(s). The petroleum ether layer was separated, then concentrated to 1/10 of its original volume. For heroin sample, ten ml of the

boiled mixture was added with 15 ml of chloroform to extract the heroin or its destructive compound (s). The chloroform layer was separated, and concentrated to 1/10 of its original volume.

TLC was used as a method for detecting either the narcotics or its destructive compounds<sup>(11)</sup>. Each sample was separately spotted at the base line across the chromatoplate (20x20cm coated with 0.5 mm of silica gel G-60 containing 0.6% starch-Merck). The samples were migrated across the plate using a solvent system of chloroform:benzene (7:3) as a running solution, and the developing reagent was 1% fast blue spray (3,3'-dimethoxybenzidinedianisidine) in 45% ethanol to determine the active componenets of hashish, wheras of heroin, the running solution was chloroform: methanol (9:1 v/v), and the developing reagent was iodoplatinate (0.25g of platinic chloride and 5g of potassium iodide were dissolved in 100 ml distilled water). The plate was allowed to dry and the separated spots can be seen under the visible light.

## **2.4 Detection narcotics material by Gas chromatography (GC)**

### **2.4.1 Detection of hashish and its destructive constituents by GC<sup>(12)</sup>**

Samples of the standard and the extract of destructive hasish as previously described in section 2.2 and 2.3 and, were injected into the GC (aliquot of 5  $\mu$ l). Detection was performed for 10 min at 100-280°C, using GC-FID detector. In addition, samples resulting from Nicloux destruction were also analyzed by the GC-FID as described above.

### **2.4.2 Detection of heroin/morphine and its destructive constituents by GC<sup>(12-14)</sup>**

Samples of standards and the extracts of destructive heroin/morphine as previously described in section 2.2 and 2.3 were injected into the GC (aliquot of 5  $\mu$ l). Detection was performed for 10min at 270°C, using GC-FID detector.

### **2.4.3 GC detection of morphine after hydrolyzing heroin by HCl**

Sample of heroin extract which hydrolyzed by 10% HCl as described in section 2.8, was injected into the GC-FID to determine the presence of morphine.

## **2.5 Determination of the optimum destructive conditions of narcotics**

### **2.5.1 Optimum concentration**

Sample of hashish (0.5 g) or heroin (100mg) were put to flask (1) (Fig.1), then 50 ml of nitric acid were added at different concentrations (4, 5, 10 and 15% in distilled water, respectively). Flasks (1), (2), (3) were connected as shown in (Fig. 1). The mixture was boiled for a fixed time (10 min), cooled to room temperature and then extracted with chloroform for heroin or with petroleum ether for hashish samples. Then worked up as described in section (2.2) and detected by TLC as described in section (2.3).

### **2.5.2 The optimum boiling time was caused complete destruction**

Sample of hashish (0.5 g) or heroin (100mg) was mixed with 5% nitric acid. The mixture was boiled for different periods of time (5,10,15 min), then processed as described in section (2.2) and detected by TLC as described in section (2.3).

### **2.5.3 Optimum quantitation of nitric acid used as destructive reagent**

Sample of hashish (0.5 g) were used in this experiment, where it has boiled in 50ml of 0.49N nitric acid (equivalent to 5% of nitric acid) for 5 min. The volume of nitric acid was filtered off and brought back to 50 ml by adding distilled water. Then 1ml was titrated against 0.1N NaOH. (Phenolphthaline indicator(ph.ph) (1% phenolphthaline in ethyl alcohol) determined the end point titration when the red colour of the indicator disappeared. The quantity of 0.1N NaOH was recorded to calculate the remaining of nitric acid in the boiling mixture and how much of the acid was reacted (consumed) with the narcotic sample.

## **2.6 Detection of hashish's active ingredients that were might trap by petroleum ether in flask (3)**

Sample from flask (3) was taken and analyzed by TLC as described in section (2.3) to detect any active components of hashish was trapped in petroleum ether.

## 2.7 Nicloux reagent (oxidizing agent) <sup>(15)</sup>

Sample of hashish (0.5 g) was extracted by shaking with 5ml petroleum ether, then the extract was filtered and transferred to a separating funnel. Nicloux reagent (6 ml) [1ml potassium dichromate (2%) and (5 ml) concentrated sulfuric acid] were added to 5 ml of the extracted sample. The mixture was gently shaken for 1min; where two layers were separated. The upper layer contained the treated extract while the lower contained a mixture of  $K_2SO_4$  and  $Cr_2(SO_4)_3$  resulted from the oxidation reaction between the components exist in the two layers. The positive oxidation reaction indicated by changing the orange colour in the lower layer to a green-blue. The petroleum ether layer was analyzed by TLC to detect whether the hashish active compounds are still active or destructed (oxidized).

## 2.8 Treatment of heroin

Heroin sample (100 mg) was mixed with 20 ml hydrochloric acid at different concentrations (5, 10, and 15 %). Each sample was boiled for 15, 30, 45 min. to determine the optimum condition for the complete conversion of heroin to morphine. In each case, the sample was extracted by shaking with 30 ml diethyl ether in a separating funnel for 10 min. to exclude any impurities in the acid media. The diethyl ether layer was discarded with the impurities while the aqueous solution containing the morphine and other alkaloids was transformed to alkaline medium by adding 1N sodium hydroxide drop by drop to precipitate all alkaloids leaving the morphine soluble in the aqueous solution as sodium morphinate. Then the solution was shaken for 10 min. by chloroform. The chloroform layer was discarded contain alkaloids other than morphine. Hydrochloric acid (10 %) was added drop by drop to acidify the aqueous solution containing the morphine. Then a saturated ammonium solution was added to the aqueous layer drop by drop until it becomes alkaline (pH 9).

The morphine was extracted by adding two volumes of chloroform: ethanol (3:1 v/v) to one volume of the aqueous solution, then the mixture was shaken in a separating funnel for 10 min. The bottom layer was transferred to a clean rotary evaporating flask connected to a vacuum pump and evaporation was

done at 78°C. The solvent was partially evaporated leaving the concentrated morphine in a small amount of the solvent which was transferred to clean vial. TLC was used to detect the active morphine.

## **2.9 Toxicological methods**

### **2.9.1 Experimental animals**

The present study was performed on male Swiss albino mice weighing from 20-26 g, obtained from Institute of Graduate Studies and Research, Alexandria University, Alexandria, Egypt. The animals were caged in group of 5-7 animals per cage. All animals were maintained on food and water *ad libitum*, except the 24 hours before decapitation. The total number of experimental animals, which has been used in this study, was 50 mice. Male mice received hashish and heroin in corn oil and other groups received extracts of degraded hashish and heroin in corn oil as a single dose for 24 h and also as repeated doses for four consecutive days at 10 mg/kg body weight. Control animals received the same volume of vehicle (corn oil) and assayed together with the treated groups. The effect of hashish and heroin and their degrading materials resulted from destructing hashish or heroin, on the experimental animals were determined.

### **2.9.2 Preparation of microsomes**

Mice were fasted overnight before decapitation, and sacrificed by exsanguination. The abdominal cavities were opened immediately and the livers were removed, washed with cold 0.1M potassium phosphate buffer, pH 4.7, blotted dry, weighed and chilled on ice. All the following operations were carried out at 4°C. A 33% (w/v) crude homogenate was prepared (1 g liver + 3 volumes of 0.1M phosphate buffer, pH 7.4) by homogenization with teflon piston using five to eight strokes. The crude homogenate was centrifuged at 11,000 xg for 20 min. at 4°C to remove intact cells, nuclei, and mitochondria. The supernatant solution was subsequently centrifuged at 105,000 xg for 60 minutes at 4°C to sediment microsomal pellets. The microsomal pellets were resuspended in 0.1 M phosphate buffer, pH 4.7, kept in ice bath and used as

the enzyme source for determination the following parameters levels:

- 1-Estimation of cytochrome P-450 according to the method of Omura and Sato<sup>(16)</sup>.
- 2-Estimation of cytochrome b-5 was determined according to the method of Omura and Sato<sup>(16)</sup>.
- 3-Assay of DMN-N-demethylase I activity using the method of Mostafa, MH, Weisburger EK.<sup>(17)</sup>
- 4-Assay of aryl hydrocarbon hydroxylase (AHH) using the methods according to of Wieble and Gelboin<sup>(19)</sup>.
- 5-Determination of the hepatic content of glutathione determined according to the method of Mitchell *etal.*<sup>(20)</sup>.
- 6-Assay of glutathione-S-transferase activity was assayed according to the method of Chi-Yu *et al.*<sup>(21)</sup>
- 7- Estimation of protein concentration in the hepatic microsomal fraction was determined according to the method of Lowry *et al*<sup>(22)</sup>
- 8-Determination of microsomal thiobarbituric acid-reactive substances (TBARS) according to Tappel and Zalkon<sup>(23)</sup>.

### 3. RESULTS AND DISCUSSION

#### Biological destruction using bacterial isolates

Bacteria were isolated from a rotten hashish material on a nutrient agar (*Clostridium cellulolyticum* sp). The isolated bacteria were tested to utilize the active component in the hashish material, by growing such bacteria at 30° C for 5 days on a minimal medium containing 1% of the extracted active material of hashish as the sole carbon source in the medium. Non of the tested bacteria was able to grow on such medium, indicating a negative result of using such method to destruct hashish. This result indicated that the isolated bacteria were possibly maintained their growth or activity on the other ingredients of hashish materials that contain plant homogenate rich with carbohydrates, protein and fats.

(a) Narcotics destruction

Determination of the best destructive reagent for hashish and heroin

Four reagents (HCl, NaOH, H<sub>2</sub>SO<sub>4</sub>, and HNO<sub>3</sub>) at 10% concentration were used to determine which have the ability to degrade either hashish or heroin. Only nitric acid was able to destruct hashish and heroin completely (Plate 1&2, Lane 4), where active ingredients of hashish had developed on the TLC chromatogram after treatment with other reagents (Lane 1-3) comparing the control (Lane 5).

Determination of the optimum concentration of nitric acid and time of boiling to destruct hashish and heroin

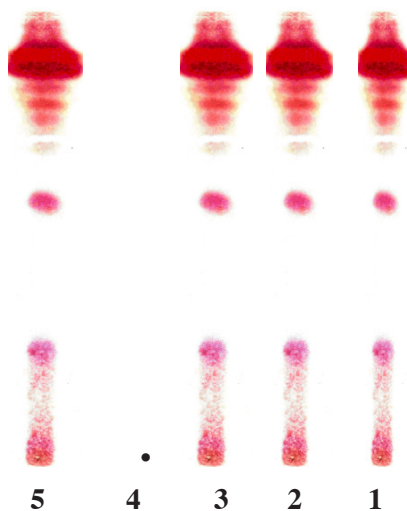
Nitric acid at 5% concentration for 5 min. was the minimum acid concentration producing complete destruction of hashish and heroin (Plate 3, 4, Lane 2), compared to the control (Lane 5), while the optimum time of boiling required to obtain complete destruction of either hashish or heroin material by 5% HNO<sub>3</sub> about 5 min. boiling was enough to obtain complete degradation of hashish or heroin. These results were confirmed by GC chromatograms as in (Fig. 6,7).

Destruction of hashish by using Nicloux reagent

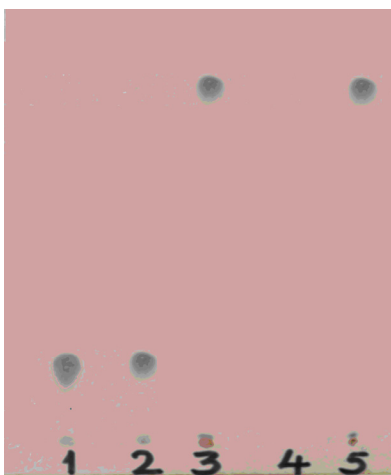
Results showed that the active ingredients of degraded hashish by using Nicloux reagent as oxidative reagent were completely destructed (Plate 5, Lane 2) while the active ingredients of hashish in the control sample was developed on TLC chromatogram (Lane 1).

Determining the optimum quantity of HNO<sub>3</sub> required to destruct hashish completely

The quantity of 5% HNO<sub>3</sub> required complete destruction of 5 grams hashish was 0.282 ml. Therefore, 1 ton of hashish required 56.56 liters of 5% HNO<sub>3</sub> which equivalent to 2.828 liters of concentrated nitric acid.

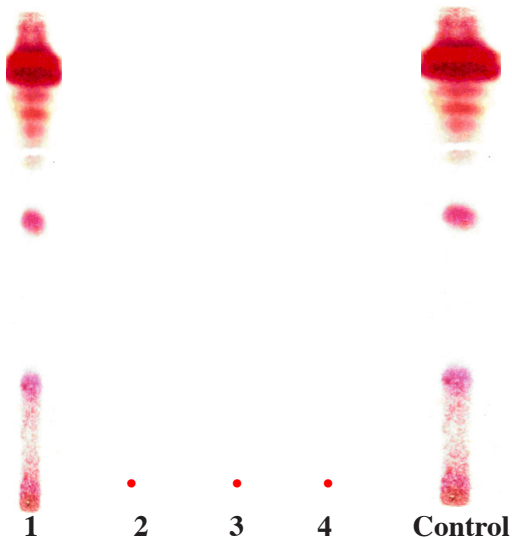


**Figure (1):** TLC chromatogram showing results of using different destructive chemical reagents with hashish , where hashish sample (0.5g) was boiled for 10 min in (1) HCl (10%), (2) NaOH (10%), (3) H<sub>2</sub>SO<sub>4</sub> (10%), (4) HNO<sub>3</sub> (10%), (5) Control. The mobile system was chloroform: benzene (7:3), and the TLC plate was developed with fast blue as developing reagent.



**Figure (2):** TLC chromatogram showing results of using different destructive chemical reagents, where heroin sample (0.1g) was boiled for 10 min. in the following reagents (1) HCl (10%), (2) NaOH (10%), (3)

H<sub>2</sub>SO<sub>4</sub> (10%), (4) HNO<sub>3</sub> (10%), (5) Control. The mobile system was chloroform: methanol (9:1), and TLC plate was developed by Iodoplatinate as developing reagent (0.25 g of platinum chloride and 5g of potassium iodide were dissolved in 100 ml H<sub>2</sub>O).



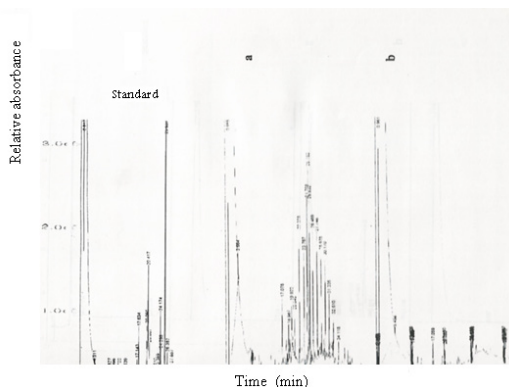
**Figure (3):** TLC chromatogram showing results of optimizing concentrations of HNO<sub>3</sub> (The best destructive reagent) for treating hashish: (1) HNO<sub>3</sub> (4%), (2) HNO<sub>3</sub> (5%), (3) HNO<sub>3</sub> (10%), (4) HNO<sub>3</sub> (15%), (5) Control. The mobile system was chloroform: benzene (7:3), and the TLC plate was developed with fast blue as developing reagent.



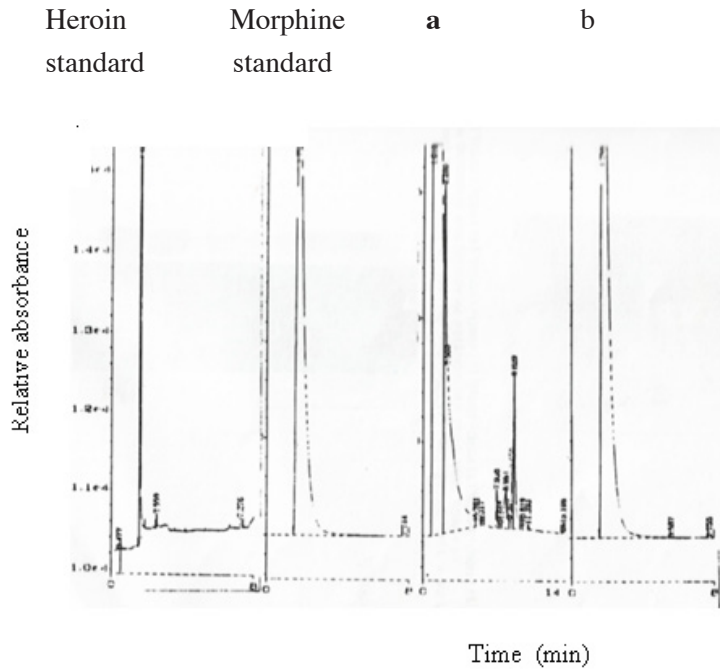
**Figure (4):** TLC chromatogram showing results of optimizing concentrations of  $\text{HNO}_3$  (The best destructive reagent) for treating heroin: (1)  $\text{HNO}_3$  (4%), (2)  $\text{HNO}_3$  (5%), (3)  $\text{HNO}_3$  (10%), (4)  $\text{HNO}_3$  (15%), (5) Control. The mobile system was chloroform: methanol (9:1), and the TLC plate was developed with Iodoplatinate as developing reagent.



**Figure (5):** TLC chromatogram showing results of using Nicloux reagent with hashish sample (0.5g) for 2 minutes (1) Control, (2) Treated sample. The mobile system was chloroform: benzene (7:3) and TLC was developed with fast blue as developing reagent.



**Figure 6:** GC chromatogram of hashish standard and compounds resulted from treating hashish by 5% nitric acid (a) and by Nicloux reagent (b)



**Figure 7: GC chromatogram of heroin and morphine standard samples and compounds resulted from treating heroin by 5% nitric acid (a) and by 10% HCL (b)**

(b) Drug metabolizing enzymes

The biotransformation can occur in many of several tissues and organs. By far the greatest number of the chemical reactions are carried out in the liver, which metabolites not only drugs but also most of the other foreign compounds to in which the body is exposed. Biotransformation in the liver is therefore, a critical factor not only in drug therapy but also in defending the body against the toxic effects of a wide variety of environmental chemicals such as insecticides, mutagens and carcinogens<sup>(24-27)</sup>. The metabolism of most of these agents involves an oxidation-reduction reaction mediated by the enzyme complex localized in endoplasmic reticulum of the liver microsomes. Conservatively, It is likly to assume that, reasonable to suggest that the vast majority of foreign organic molecules are metabolized by the mammalian microsomal enzyme system<sup>(28,29)</sup>.

Our study showed that:

- (a) single- and repeated-dose treatments of mice with hashish increased:
- (1) the hepatic content of CYP450 by 112 and 206%, respectively;
  - (2) NDMA-dI activity by 23 and 41%, respectively;
  - (3) AHH activity by 110 and 165%, respectively;
  - (4) NADPH\_ cytochrome c reductase activity by 21 and 98%, respectively;
  - (5) glutathione level by 81 and 173%, respectively (Table 1). In addition, GST activity increased only after repeated-dose treatments of mice with hashish (Table 1). Contrarily to the effects of hashish on the activity of the above mentioned enzymes, the present study showed that single-dose treatment of mice with heroin increased the hepatic content of CYP450, AHH activity, NADPH-cytochrome c reductase, glutathione level by 126, 72, 39 and 205%, respectively (Table 2). However, repeated-dose treatments of mice with heroin did not change the activity of most enzymes except NADPH\_ cytochrome c reductase activity increased by 20% (Table 2). On the other hand, the level of free radical was markedly decreased after single- and repeated-dose treatments of mice with either hashish or heroin (Tables 1 and 2). In addition, the present study showed the expression of CYP 2E1 which induced in liver microsomes of mice after administration of hashish as single- and/or repeated-doses, whereas such expression decreased after heroin treatment. Moreover, single and repeated dose treatments of mice with hashish was found to induce the expression of CYP 2C6. On the other hand, the expression of CYP2C6 was potentially reduced after repeated dose.

On the other hand, there was a decrease in levels of TBARS in single and repeated dose treatments of mice with hashish by 76% and 78%, respectively and in single and repeated dose treatments of mice with heroin by 85% and 71%, respectively.

The carcinogenic potency of benzo(a)pyrene (B(a)P) and the extent of binding of its ultimate metabolites to proteins and DNA has been correlated with the induction of AHH activity<sup>(30)</sup>.

In accordance with this observation, high incidence of lung cancer among hashishism was found in Casablanca, Morocco<sup>(31)</sup>, and this might be due to the presence of high levels of PAHs, e.g. B(a)P, in tobacco smoke<sup>(32)</sup>. In the present study, the hepatic content of CYP, AHH, and NADPH cytochrome c reductase activities were potentially induced after single and repeated-dose treatments of mice with hashish (Table 1), and also after single-dose treatment with heroin (Table 2). In agreement with our finding, it has been found that dietary oral intake of petroleum ether extract of cannabis increased the activity of AHH and also CYP in the liver of rats<sup>(33)</sup>. In addition, marijuana induced higher levels of AHH dependent-CYP 1A1 than did tobacco tar, and this induction was primarily due to delta-(9)-tetrahydrocannabinol (delta-(9)-THC), the psychoactive component of hashish<sup>(32,33)</sup>. Moreover, it has been found that delta-(9)-THC acts through the aromatic hydrocarbon (Ah) receptor to activate transcription of CYP mRNA, and the percentage of CYP induction increased with increasing the concentration of delta-(9)-THC<sup>(34)</sup>. Variations in the activity of CYP isozymes are well correlated with deleterious effects of chemical carcinogens, such as N-nitrosamines, which are primarily metabolized in the liver by hepatic microsomal NDMA-dI and CYP2E1<sup>(35,36)</sup>. In the present study, NDMA-dI activity and the expression of CYP 2E1 were markedly induced in liver microsomes of mice after administration of hashish as single- and/or repeated-doses (Table 1), whereas such activity and 2E1 expression were decreased after heroin treatment after single dose treatment (Table 2). In accordance with the present study, it has been found that dietary oral intake of petroleum ether extract of cannabis increased the activity of aminopyrene N-demethylase in the liver of rats<sup>(37)</sup>. It is well known that induction of NDMA dI activity could lead to an increased production of reactive alkylating agents that could bind to DNA and other macromolecules. Therefore, the deleterious effects of N-nitrosamines might be increased in the liver and probably other organs of hashish smokers<sup>(37)</sup>.

Interestingly, the level of free radical was markedly decreased

after single- and repeated-dose treatments of mice with either hashish or heroin (Tables 1 and 2). Therefore, the mechanism of hashish and heroin toxicity could be related to induction of drug-metabolizing enzymes and not to generation of free radical.

- (b) single- and repeated-dose treatments of mice with degraded hashish and heroin : (1) There was an increase of hepatic P450 activity in single and repeated doses of degraded and heroin by 31% and 104%, respectively and in repeated dose of hashish by 67% (2) There was an increase of hepatic glutathione activity in single dose of degraded hashish and heroin by 142% and 83%, respectively and an increase in repeated dose of heroin by 139% , while there was no change in the activity of glutathione in repeated dose of degraded hashish and there was an increase in single dose of heroin and in repeated dose of hashish and heroin by 31%, 67% and 104%, respectively. (3) There was no significant change in the activities of AHH, DMN-N demethylase, Cytochrome b-5, GST in single and repeated dose treatments mice and in the levels of TBARS in single and repeated doses of degraded hashish. On the other hand there was a decrease in the levels of TBARS in single and repeated dose treatments of degraded heroin by 81% and 79%, respectively.

The effect of degrading materials (resulted from destructing hashish or heroin), on the experimental animals (male mice) were determined. The materials were orally administered into the experimental animals either in a single or in a repeated dose. Generally, the results of these experiments showed that the liver microsomal enzymes did not significantly affected. This indicated that those materials have no toxic effect on the liver cells. On the other hand, the degraded materials of hashish and heroin increased the level of cytochrome P450 with the repeated dose and with the single dose in case of Glutathione, which is considered a defensive mechanism for detoxification of many toxic compounds.

#### 4. Conclusion

- (1) Treatment study concluded that degradation of hashish and heroin by nitric acid is an economic, safe, and clean method. In general, the results of these experiments showed that the liver microsomal enzymes did not significantly affected. This indicated that those degraded materials not only have no toxic effect on the liver cells but also they considered a defensive mechanism for detoxification of many toxic compounds.
- (2) Moreover, the present study demonstrates alterations in the cellular levels of CYP isozymes that are responsible for the bioactivation of various carcinogens, xenobiotics, and also steroid hormones. Differences in activities of drug-metabolizing enzymes may alter the bioavailability or efficacy of drugs, and increase toxicity of certain xenobiotics and others environmental compounds. Changes in CYP isozymes expression, under the influence of hashish or heroin, may directly produce inter individual differences in susceptibility to carcinogenic compounds which toxicity is modulated by these enzymes. In addition, such alterations may also change the therapeutic actions of drugs which are primarily metabolized by the P450 system and also such changes should be considered when therapeutic agents are administered to peoples addicted hashish or heroin.

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Table 1: Influence of hashish /hashish degraded after single- and repeated-dose treatments on the activity of drug-metabolizing enzymes in the liver of male mice

Enzymes& Tests	Control	Single dose of Degraded hashish		Single dose of standared hashish		Repeated dose of degraded hashish		Repeated dose of standared hashish	
		Mean	% Change	Mean	% Change	Mean	% Change	Mean	% Change
P <sub>450</sub>	1.83±.15	1.66±.2	NE	3.87±.31	112%	3.05±.19	67%	5.58±.53	206%
AHH	123±4.5	126±8.9	NE	258±19.5	110%	131±8.7	NE	326±23.6	165%
DMN-N demethylase	177±9.2	180±10.7	NE	218±19.9	23%	182±12.6	NE	249±17.4	41%
Glutathione	8.2±.81	19.9±7.9	142%	14.9±1.1	81%	6.96±.64	NE	22.4±2.4	173%
GST	.59±.05	0.57±.04	NE	.52±.04	NE	.65±.073	NE	1.31±.1	75%
b-5	1.78±.08	1.83±.12	NE	1.48±.09	NE	1.54±.29	NE	2.48±.13	39%
TBARS	.39±.07	0.43±.06	NE	.093±.01	-67%	.41±.09	NE	.085±.01	-78%

Table 2: Influence of heroin/heroin degraded after single- and repeated-dose treatments on the activity of drug-metabolizing enzymes in the liver of male mice

Enzymes& Tests	Control	Single dose of Degraded heroin		Single dose of standared heroin		Repeated dose of degraded heroin		Repeated dose of standared heroin	
		Mean	% Change	Mean	% Change	Mean	% Change	Mean	% Change
P <sup>450</sup>	1.83±.14	2.39±.15	31%	4.12±.34	126%	3.62±.41	104%	1.76±.15	NE
AHH	123±4.5	147±8.4	NE	212±20.4	72%	105±25.9	NE	120±12.9	NE
DMN-N demethylase	177±9.2	163±12.4	NE	126±3.2	-29%	167±18.4	NE	143±9.2	NE
Glutathione	8.2±.81	15.7±2.4	83%	25±1.78	205%	19.6±.66	139%	8.4±1.6	NE
GST	.59±.05	.49±.22	NE	.51±.07	NE	.53±.07	NE	.56±.03	NE
b-5	1.78±.08	1.83±.14	NE	1.56±.09	NE	1.62±.07	NE	1.58±.12	NE
TBARS	.39±.07	.07±.005	-81%	.048±.01	-85%	.08±.018	-79%	.115±.02	-71%

