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Toxicity study of confiscated illicit opium and heroin on liver

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Abstract
The impact effect of common narcotic drugs (Heroin & Opium), in comparison with experimentally proven hepatotoxic agents (carbon tetrachloride (CCl_4), on liver of mice was assessed. Histopathological investigations of the liver sections of revealed abnormalities of the liver tissues (extensive infiltration of hepatocytes and inflammation of portal tract, dilated blood sinusoids with connective tissue proliferation in the portal areas, wide areas of hepatocellular necrosis, and periportal fibrotic formation with thick septa) indicating liver hepatotoxicity. Illegally sold opium samples is usually adulterated and contaminated with high level of arsenic and lead that may be associated with liver diseases, so the level of arsenic and lead in illicit opium and heroin samples was determined using atomic absorption method. This is an attempt to define the incidence and severity of liver disorders among a large number of drug addicts. The concentration of arsenic and lead were found to be high in illicit opium and heroin samples. *In conclusion:* The hepatotoxicity caused by the administration of the common narcotic drugs (illicit opium and heroin samples) may be partially due to excessive cumulative doses of illicit narcotic drugs, and/or the presence of heavy metals (arsenic and lead).

Keywords: narcotic drugs, heroin, opium, liver cell damage, hepatotoxic agents, carbon tetrachloride, histopathological investigation, atomic absorption, lead, arsenic

1. Introduction
Acute and chronic liver diseases were reported among heroin addicts, laboratory evidence of hepatic dysfunction has been reported in up to 75 % of parenteral heroin users. The cause has usually been related to viral hepatitis. Other factors, such as the effect of heroin adulterant mixtures and multiple drug abuse upon the liver tissues have also been implicated. In contrast, little attempt is given to define the incidence and severity of liver abnormalities in a large group of non-parenteral drug abuse [1], so the impact of illicit opium and heroin samples, in comparison with experimentally proven hepatotoxic agent (CCl_4) on the liver of mice was assessed.
Cases with acute lead poisoning due to contaminated opium were reported. The predominant features of most cases were: hepatic failure, reversible acute tubular necrosis, severe neuropathy and respiratory paralysis. Chelation therapy resulted in a fall in blood lead to within normal limits [2-4].
Illegally sold opium is usually adulterated with high level of arsenic. This adulteration was supposed to enhance the aphrodiasic proporties of opium. Some patients, who are opium addicts, may have a clinical picture of arsenical neuropathy and hepatomegaly. Elevated levels of arsenic may be associated with liver disease. Microscopic examination of liver tissues revealed periportal fibrosis. The opium obtained from the Indian source revealed to have an exceptionally high arsenic content (25mg/100gm) [5-9]. Therefore the level of lead and arsenic were measured in the confiscated opium and heroin samples.

2. Experimental
2.1 Materials and Reagents
2.1.1 Drugs
***Confiscated heroin sample*:** It was obtained from seizure number 978/1988, Suez- Egypt. It was formed of cylindrical pieces with rounded ends, 20 cm L and 4 cm D. Each piece was wrapped externally with green adhesive tape, 1.5 cm W, and internally another wrapping of yellowish-white to light brown paper. After the removal of wrapping, heroin was found in the form of small hard granular pieces, grayish-brown to dark brown to nearly blackish in color, measuring about 0.5-1.5 cm D and having intense vinegar like odor. Grinding of heroin pieces produces a light brown to dark brown powder with repulsive vinegar-like odor.

***Confiscated opium sample*:** It was obtained from seizure number 422/1991, Ataka, Suez-Egypt. It occurred in the form of large rectangular blocks wrapped in yellowish-white papers, measuring about 15 cm L, 10 cm W and 10 cm H. After removing of the wrapping the blocks appeared black in color and having a strong characteristic narcotic odor. From one of the opium blocks a piece of 50 gm was removed and powdered in a glass mortar to produce a dark brown to nearly black powder.

2.1.2 Chemicals
All chemicals and solvents are of analytical grade and purchased from Adwik, Egypt.

2.1.3 Reagents
Histopathological reagents: Hematoxylin and Eosin (Hx and E), Phosphotungstic acid hematoxylin *(PTAH)* and Masson,s trichrome (MT) stains.

2.1.4 Apparatus
Microtome (Leica R M 2025, Nussloch); Digital camera Olympus 2020 fitted with C mount (Olympus company, Japan); Atomic Absorption spectrophotometer, Varian SpectrAA 220, with air-acetylene flame for lead and graphite for arsenic at National Research Center, Dokki, Cairo, Egypt.

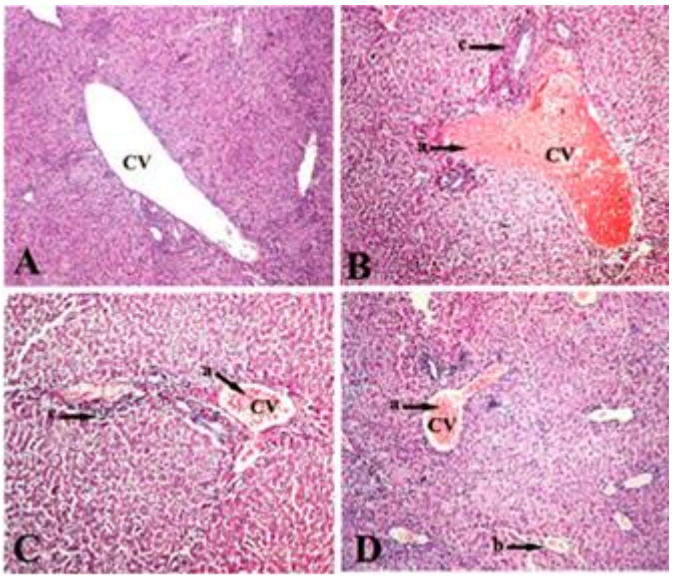
2.1.5 Experimental animals
Male albino mice (local bread, 20-25 gm), were purchased from Theodor Billharze Institute, Cairo, Egypt.

2.2 General Procedure
2.2.1 Histopathological investigation
2.2.1.1 Animal grouping
Fifty mice were divided into ten groups (five mice each).
Group 1 (control): Injection of 200µl normal saline I.P 3 doses / 3 days intervals.
Group 2-5: Injection of 5 mg / ml fresh opium in saline solution I.P 3 doses / 3 days intervals in a dose equals to 25, 50, 75 and 100 mg/kg body weight.
Group 6-9: Injection of 5 mg / ml fresh heroin in saline solution I.P 3 doses / 3 days intervals in a dose equals to 25, 50, 75 and 100 mg/kg body weight.
Group 10: Injection of a fresh mixture of CCl_4 and corn oil (equal volumes) I.P. 3 doses / 3 days intervals in a dose equals to 2 ml /kg body weight.

2.2.1.2 Samples Collection
All these animal groups were under experiment for 10 days. Animals were scarified by cervical dislocation, 24hr after the last dose, livers were immediately excised, fixed in 10% neutral buffered formalin pH 7.4, dehydrated in a ascending grades of absolute ethyl alcohol. Cleared in xylene and then embedded in paraffin wax and sectioned to 4µm thickness. For histopathological studies, sections were stained with hematoxyline and Eosin (Hx and E). For histochemical studies, sections were stained with phosphotungistic acid hematoxylin and Masson·s trichrome. All sections were examined by light microscope (x 10) to examine the histopathological changes.

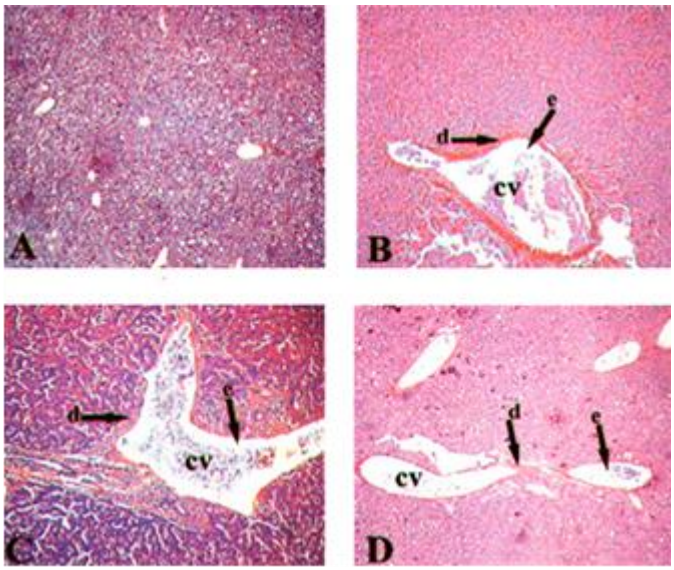
2.2.2 Determination of arsenic and lead in illicit opium and heroin samples.
Sample preparation
Illicit opium and heroin stock solutions were prepared at conc. 5 mg / ml distilled water.
Determination of arsenic and lead
The level of arsenic and lead in illicit opium and heroin samples was determined using atomic absorption method.

3. Results and discussion
3.1 Results
3.1.1 Histopathological investigation
Histopathological examination of liver tissues revealed pericentral inflammation and diffused fatty changes. Staining with hematoxylin and Eosin showed extensive infiltration of hepatocytes and inflammation of portal tract and dilated blood sinusoids (Figure 1). Staining with phosphotungstic acid hematoxylin, showed prefibrotic changes due to excessive changes of CCl4 and / or illicit samples and mild dilation of central vein (Figure 2). Staining with Masson's trichrome (Figure 3), showed: Collagen fiber around the wall of central vein, hepatic artery, Portal vein and bile duct of portal tract. Collagen fiber in interstitial spaces. Progressive periportal fibrosis. Mild dilation of central vein.



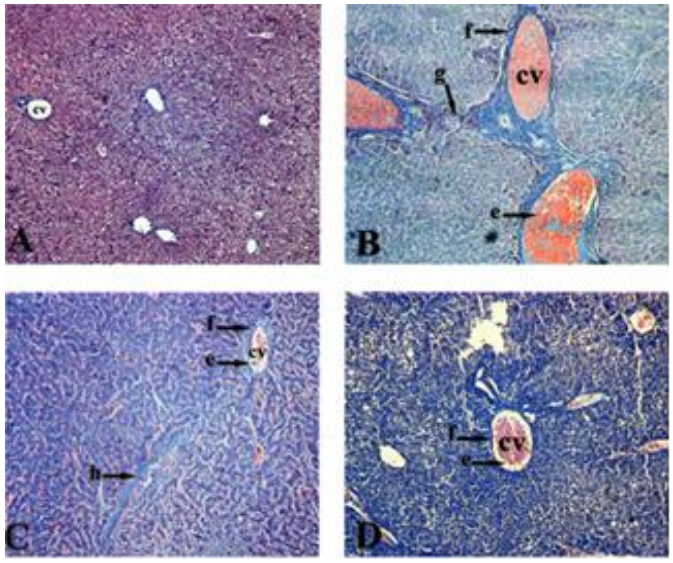
c.v. = Central vein, b = dilated blood sinusoids, a = extensive infiltration of hepatocytes by mononucleal leukocytes and inflammation of portal tract, C = inflammatory cell

Fig 1: Photomicrograph of liver of control mice (A), those received heroin (B), opium (C), and CCL4 (D) stained with Hx & E, x10.



c.v. = Central vein, e = mild dilation of central vein, d = Prefibrotic changes due to excessive changes of CCL4 and / or illicit samples

Fig 2: Photomicrograph of liver of control mice (A), those received heroin (B), opium (C), and CCL4 (D) stained with (Phosphotungestic acid heamatoxylin stain, x10).



c.v. = Central vein, e = mild dilation of central vein, f = collagen fiber around the wall of central vein, hepatic artery, Portal vein and bile duct of portal tract, g = collagen fibers in interstitial spaces, h = progressive periportal fibrosis

Fig 3: Photomicrograph of liver of control mice (A), those received heroin (B), opium (C), and CCL4 (D) stained with Masson's trichrome stain, x10)

3.1.2 Determination of lead and arsenic

The level of lead and arsenic in illicit opium and heroin samples is shown in table (1).

Table 1: Determination of lead and arsenic on illicit opium and heroin samples using atomic absorption method.

Metal	Illicit opium sample	Illicit heroin sample
Arsenic	143.6 mg/ 100 gm illicit opium sample	88.4 mg/ 100 gm illicit heroin sample
Lead	130 mg/ 100 gm illicit opium sample	128 mg/ 100 gm illicit heroin sample

3.2 Discussion

3.2.1 Histopathological investigation

Carbon tetrachloride induced hepatotoxicity has been widely used as an animal model to study liver injury. CCl4 is biotransformed by cytochrome P-450 in the hepatic microsomal oxidase system to trichloromethyl free radicals [10, 11]. The reactive CCl_3 radicals bind to unsaturated fatty acids in the cytoplasmic membrane and induce lipid-peroxidation. This may alter intracellular calcium homeostasis or impair protein biosynthesis and finally cause cell death [11-14].

The histopathological abnormalities of the liver tissues (pericentral inflammation, acute inflammatory changes with connective tissue proliferation in the portal areas, diffused fatty change, wide areas of hepatocellular necrosis, and pseudo-lobular fibrotic formation with thick septa) in opium and heroin treated groups proved their hepatotoxicity. These results are in agreements with the reported hepatotoxicity of opium and heroin which starts from chronic hepatitis to fibrosis, necrosis and finally liver failure [15, 16].

3.2.2 Determination of lead and arsenic

i) Arsenic

Opium obtained from government sources had 0 to 18.2 µg of arsenic per 100 gm of opium [6]. The high arsenic content of illicit opium (143.6 mg/ 100 gm) sample and illicit heroin (88.4 mg/ 100 gm) sample confirms that the provided illicit opium sample is adulterated with arsenic. The use of arsenic can affect the liver adversely and has been well documented as an etiologic agent for the development of cirrhosis and idiopathic portal hypertension. Available information reveals that the so called opium manufacturers adulterate it with arsenic in varying quantity for two reasons; firstly it is believed to be a general tonic and secondly it is said to be an aphrodisiac, which, when combined with opium - enhances the aphrodisiac quality of opium. So opium or heroin addicts will have a clinical picture of arsenical neuropathy and hepatomegaly [6].

ii) Lead

The concentrations of lead in various food items are highly variable. Several studies have reported average lead intakes in the range of 100–500 µg/day for adults, with individual diets covering a much greater range. More recent data indicates total daily intakes of about 100 µg or less [17], 1000 µg / g lead in soil or dust [18] and 1.25 µg/g food [19] may cause lead toxicity. So 130 mg/ 100 gm illicit opium sample and 128 mg/ 100 gm illicit heroin sample will considered to be a high level of lead, leading to lead toxicity.

Lead-induced health effects in adults [20]

The toxicity of lead may largely be explained by its interference with different enzyme systems: lead inactivates these enzymes by binding to SH-groups of its proteins or by displacing other essential metal ions. For this reason many organs or organ systems are potential targets for lead, and a wide range of biological effects of lead have been documented. These include effects on haem biosynthesis, the nervous system, the kidneys and reproduction, and also cardiovascular, hepatic, endocrinal and gastrointestinal effects.

1. Effects on the nervous system may lead to encephalopathy signs and symptoms, peripheral nerve dysfunction (slowed nerve conduction velocities).
2. Effects on haem synthesis lead to anemia.
3. Effects on kidney function develop nephrotoxicity.
4. Effects on blood pressure causes a two-fold increase in blood lead was associated with a 1-mmHg increase in systolic and a 0.7-mmHg increase in diastolic blood pressure.

4. Conclusion

The hepatotoxicity caused by the administration of the common narcotic drugs (illicit opium and heroin samples) may be partially due to excessive cumulative doses of illicit narcotic drugs, foreign matters and the presence of heavy metals (arsenic and lead).

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