Oxidative Stress and Some Cellular Blood Variables in Morphine Addicted Female Rats

*Goran Qadir Othman, **Yasin Karem Amin
*Medical Lab. Technique Dept.- Medical Technical Institute-Technical Inst. Foundation- Erbil
** Anatomy and Histology Dept.- College of Medicine- Hawler Medical University

Abstract

Morphine belongs to a group of medicines called analgesics. It is an opioid analgesic that acts by blocking pain and it has high addiction property. Therefore, morphine is commonly abused narcotic analgesic in the world besides to heroin which is synthesized as morphine derivative and came into use. This study investigated the role of morphine addiction on lipid peroxidation and alteration of blood cells in female rats. The experimental rats divided to two groups; Control group and Morphine treated group (The rats were treated with i.v. injection (150mg/kg) morphine sulfate twice weekly for two weeks). The Blood samples were taken for assessing blood cell variables and the sera were used for Malondialdehyde (MDA) evaluation. The data revealed a significant ($P<0.01$) elevation of MDA in morphine treated rats. Slight anemia were appeared in morphine treated rats, as the level of Hb decreased significantly ($P<0.05$) and the Mean Corpuscle Volume (MCV) and Red cell Distribution Width (RDW) again slightly decreased, but non-significantly. There were significant declining of the level of Mean platelet volume (MPV) and plateletcrit (PCT) in morphine treated animals. This finding explains the adverse effects of morphine addiction on oxidation level in the body besides to its action on the structural and numerical variables of blood cells in female rats.

Key words: Morphine, Oxidative stress, Rats

Introduction

Morphine is an extremely potent opiate analgesic psychoactive drug that has a high potential for addiction; tolerance and both physical and psychological dependence develop rapidly. It is considered to be the prototypical opioid. In clinical medicine, morphine is regarded as the gold standard, or benchmark, of analgesics used to relieve severe or agonizing pain and suffering. Like other opioids, e.g. oxycodone (OxyContin, Percocet, Percodan), hydromorphone (Dilaudid, Palladone), and diacetylmorphine (heroin), morphine acts directly on the central nervous system (CNS) to relieve pain (Hiramatsu, 2010).

In controlled studies comparing the physiological and subjective effects of injected heroin and morphine in individuals formerly addicted to opiates, subjects showed no preference for one drug over the other. Equipotent, injected doses had comparable action courses, with no difference in subjects' self-rated feelings of euphoria, ambition, nervousness, relaxation, drowsiness, or sleepiness (Martin and Fraser, 1961). Short-term addiction studies by the same researchers demonstrated that tolerance developed at a similar rate to both heroin and morphine. When compared to the opioids hydromorphone, fentanyl, oxycodone, and pethidine/meperidine, former addicts showed a strong preference for heroin and

morphine, suggesting that heroin and morphine are particularly susceptible to abuse and addiction. Morphine and heroin were also much more likely to produce euphoria and other positive subjective effects when compared to these other opioids (O'Neal, 2006).

Other studies, such as the Rat Park experiments, suggest that morphine is less physically addictive than others suggest, and most studies on morphine addiction merely show that "severely distressed animals, like severely distressed people, will relieve their distress pharmacologically if they can (Weissman and Haddox, 1989). In these studies, rats with a morphine "addiction" overcome their addiction themselves when placed in decent living environments with enough space, good food, companionship, areas for exercise, and areas for privacy. More recent research has shown that an enriched environment may decrease morphine addiction in mice (Xu, et al. , 2007).

Morphine acts on a specific receptor of nerve cells. More specifically many such receptors are found in the spinal cord's substantia gelatinosa, a region where pain signals are first processed. The architecture of the morphine receptor is what dictates the morphine rule. There is a flat part that binds to the aromatic ring, a cavity that attracts the two carbon atoms and an anionic site that accommodates the tertiary nitrogen atom. When morphine or another agonist binds to the receptor, the cell membrane's affinity for sodium ion changes. This eventually reduces the release of neurotransmitters from the affected neurons. Investigators learned about morphine's mode of action by applying it and other opiates (including enkephalin) to guinea-pig intestines. (What else was gong to serve as the guinea pig for their experiments?) In the presence of antagonists, Na+ affinity was restored and intestinal contractions which had dropped precipitously shot up again (Enrico, 2004).

Morphine has been demonstrated to exert oxidative stress in various cells (Singhal et al., 1994). Moreover, morphine has been shown to up-regulate the expression of hemeoxygenase (HO), a biological marker of oxidative stress(Sharp et al. , 1985). We recently proposed that morphine may be inducing macrophage injury in mice and humans by different pathways (Kapasi et al. , 2004).

Aim of the study

We examined the role of morphine on the oxidative stress elevation and alteration of blood cell variables in morphine-induced addiction in experimental rats.

Materials and methods

Animals and housing: Ten adult male albino rats of about 250-300g body weight and 8-10 weeks old were used. Males were preferred to avoid the physiological changes associated with the oestrus cycle of females. The experiments were achieved between June 2010 – November 2010 in the animal house at the Dept. of Biology /College of Science/ University of Salahaddin -Erbil. Animals were housed in plastic cages bedded with wooden chips. During the experimental period five animals were kept in each cage and they were housed under standard laboratory conditions, 12:12 light/dark photoperiod (LD) at 22 ± 2 °C. (Coskun et al. , 2004).

Experimental Design

This experiment was conducted to test the toxic effects of morphine in female rats. The experimental rats were divided to two groups of rats and the treatments were continued for 2 weeks.

Group 1: Control: The rats of this group (5 rats) were given a standard rat chow and tap water ad libitum.

Group 2: Morphine treated rats: The rats of this group (5 rats) treated with i.p. injection of morphine (150 mg/kg b.w.) twice a week for two weeks.

Collection of blood samples

At the end of each experiment, the rats were anesthetized with ketamine hydrochloride (100mg/kg). Blood samples were taken by cardiac puncture into chilled tubes with or without ethylene diaminetetra acetic acid (EDTA) (4.5mM) as anticoagulant and centrifuged at 3000rpm under 0C for 15 minute; then serum were stored at -80C (Sony, Ultra low, Japan) (Haen,1995).

Haematological analysis

RBC count, WBC count, haemoglobin concentration (Hb), haematocrit (Hct) , platelet counts , mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR) were determined by using automated haematology analyzer (Syamex model: K-1000, Japan).

Determination of serum malondialdehyde (MDA):

The assessment of the lipid peroxidation process is done by determination of the end product, malondialdehyde (Muslih et al. , 2002). The level of serum MDA was determined spectrophotometrically, in brief , 150 µl of serum sample was mixed with 1ml trichloroacetic acid (TCA) 17.5% and 1ml of 0.66% thiobarbituric acid (TBA), then vortexed, incubated in boiling water for 15 minutes, and allowed to cool. After that one ml of 70% TCA was added. The mixture was allowed to sit at room temperature for 20 minutes. Then the sample centrifuged at 2000 rpm for 15 minutes, and the supernatant absorbency was read spectrophotometrically at 532nm wavelength.

All data were expressed as average and statistical analysis was carried out using statistically software (SPSS version 11.5).Data analysis was made using student t- test. The levels of significance were set at $P<0.05$.

Results

Data analysis revealed that morphine have ability to increase the level of lipid peroxidation significantly ($P<0.01$) through elevation of the MDA level. The value of MDA in morphine treated group was (7.35 Mmol/l) which was more than two fold of control rats MDA level (2.95Mmol/l) as illustrated in figure (1).

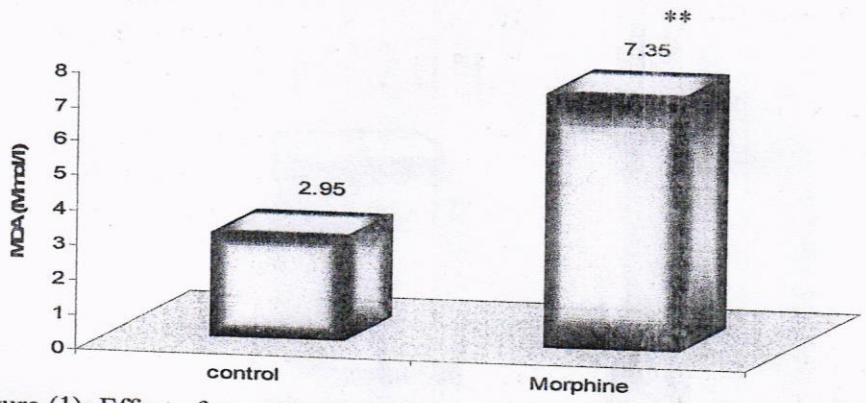

Figure (1): Effect of morphine treatment on the level of MDA in female albino rats.
** means significant at level (P<0.01)

Statistical analysis revealed a significant decrease in the level of Hb (P<0.05), with mean value reached (12.78 mg/dl) (Figure 2). While, MCV didn't affected by morphine treating, as the level of it decreased non significantly with mean (62.68), as clarified in figure (3). The current results also showed non-significant increase of RBC number in morphine treated animals comparing to control group (Figure 4).

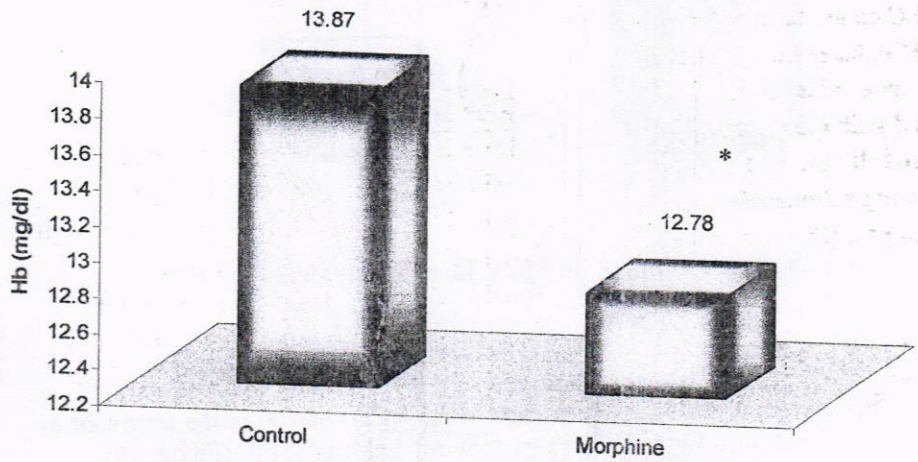

Figure (2): Effect of morphine treatment on the level of Hb in female albino rats.
* means significant at level (P<0.05)

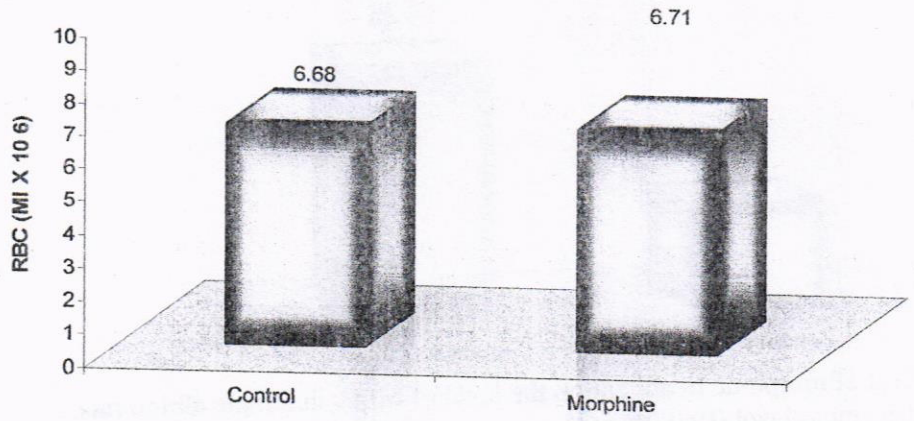

Figure (3): Effect of morphine treatment on the RBC count in female albino rats.

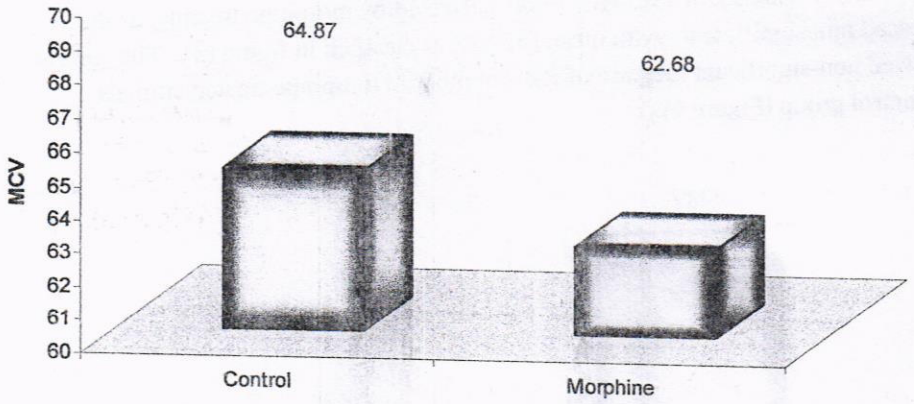

Figure (4): Effect of morphine treatment on MCV level in female albino rats.

A significant decrease ($P<0.05$) in RDW percent value was observed in the treatment of morphine to about (11.6%) comparing to (12.47%) in control diet treated rats (Figure 5).

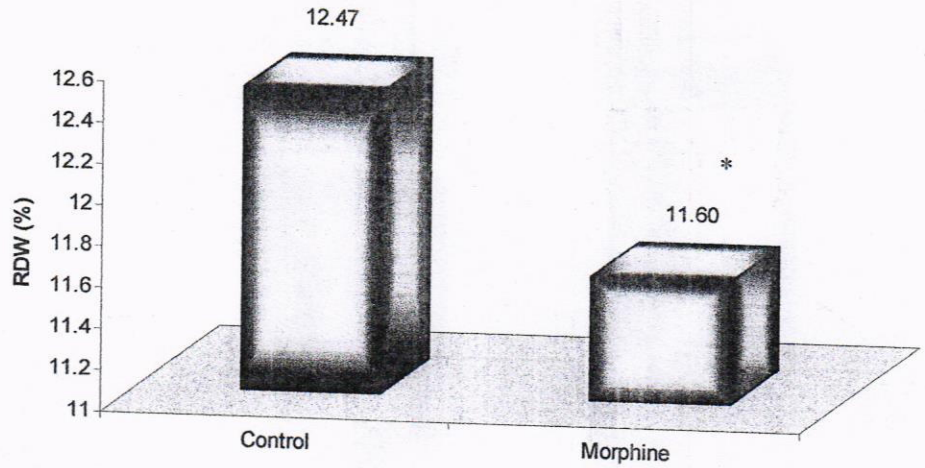

Figure (5): Effect of morphine treatment on the level of RDW in female albino rats.
* means significant at level (P<0.05)

The plateletcrit (PCT) significantly decreased (P<0.05) in morphine group with value (0.18%) as compared to control value (0.26%) as shown in figure (6). This reduction in plateletcrit was very clearly reflected in the results of MPV, as the level of MPV decreased significantly (P<0.05) in the treatment group (3.53%) according to control MPV value (5.33%) (Figure7). Meanwhile, unexpectedly, the number of platelet increased significantly (P<0.05) in rats injected with morphine (Figure 8).

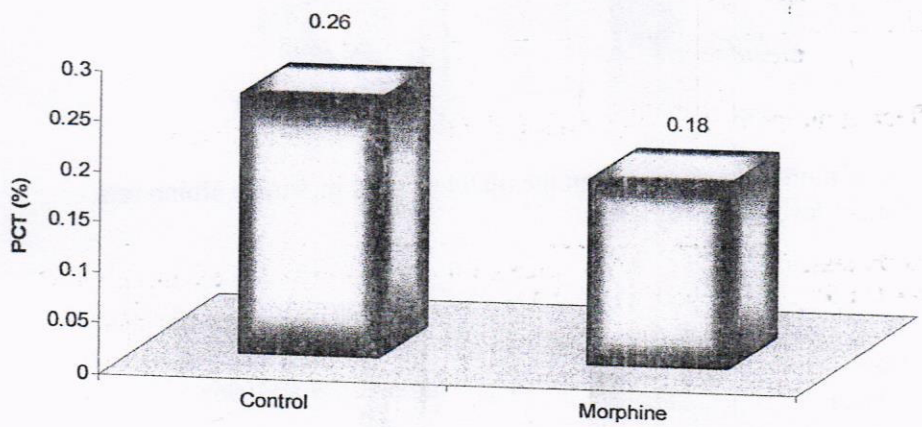

Figure (6): Effect of morphine treatment on the level of PCT in female albino rats.
* means significant at level (P<0.05)

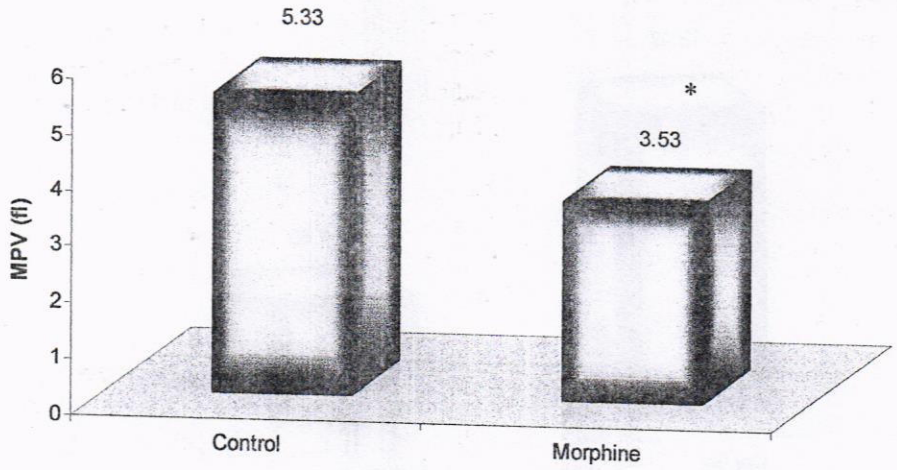

Figure (7): Effect of morphine treatment on the level of MPV in female albino rats.
* means significant at level (P<0.05)

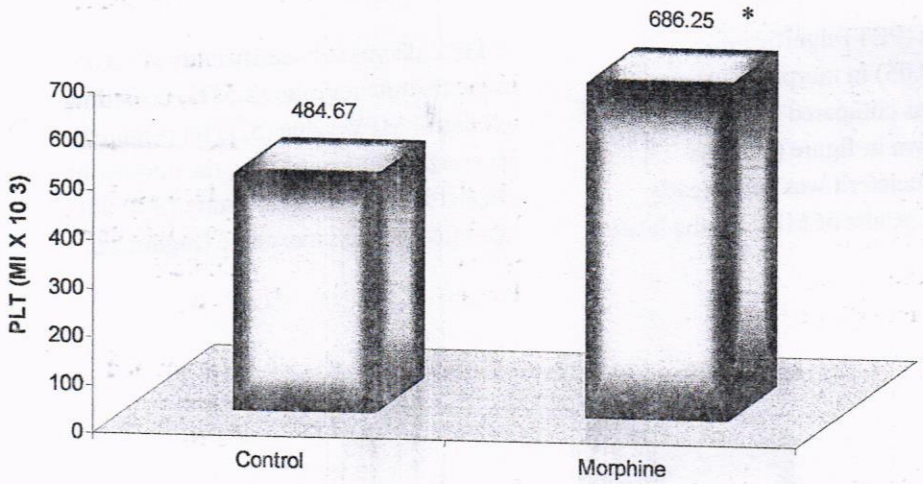

Figure (8): Effect of morphine treatment on the platelet count in female albino rats.
* means significant at level (P<0.05)

Discussion

Elevation of the MDA level can be used as indicator for oxidative stress especially related to lipid oxidation. The relation between morphine and rising of oxidative stress or depletion of antioxidants were previously studied by some researchers. Morphine showed to induce superoxide generation (Jaimita et al., 2003), induce apoptosis in fibroblasts and macrophage (Patel *et al.*, 2003) and it also has ability to inhibit glutathione (GSH) production by liver (Nagamatsu et al. 1996).

Morphine treatment tended to decrease in the level of Hb significantly , this declining didn't lead to anemia. Therefore, MCV didn't affected by morphine treating. The anemia caused by morphine is almost related to the deficiency in vitamin B12

and iron (Yunis and Casson, 2000). This unchanged volume mean of RBC is due to the non-significant increase of RBC number in morphine treated animals comparing to control group.

The slight anemic symptom in the rats were also indicated through significant decrease ($P<0.05$) in RDW percent value. The morphine addiction leads to increasing in blood viscosity, and elevation in RBC parameters are usually seen on morphine and heroine abusers (Antonova et al., 2008).

There are confusions in the results of researchers about the effect of morphine on platelets. Morphine has ability to promote platelet function through induction of platelet plug formation, thereby, it can lead to induction of thrombin formation (Hsiao et al., 2003). However unexplained immune thrombocytopenia were detected in rats treated with morphine (Cimo et al. , 1982), while an increased thrombocyte count was observed in mothers affected with morphine drug addiction (Hanssler and Roll, 1994).

Conclusion

We conclude that morphine addiction has significant negative impacts on oxidation level in the body and it also acts on the structural and numerical variables of blood cells in female rats.

Recommendation

We recommend for avoiding using of this drug in the medical treatment as analgesia unless in necessary cases.

References

1-Antonova, N.; Zvetkova,E.;Ivanov, I. and Savov, I. Hemorheological changes and characteristic parameters derived from whole blood viscometry in chronic heroine addicts. Clinical Hemorheolo. Microcire. 2008, 39(1-4):53-61.

2-Cimo, P.; Hammond, J. and Moaki, L. Morphind –induced immune thrombocytopenia. Arch. Internal Medicine.1982, 142(4):843-844.

3-Coskun, O.; Ocakci, A.; Bayraktaroglu, T.; Kanter, M. Exercise training prevents & protects streptozotocin-induced oxidative stress & beta-cell damage in rat pancreas. Tohoku. J. Exp. Med.2004, 203:145-54.

4-Enrico, U. (2004). Morphine . LaurenHill Academy. Montreal, Canada .

5-Haen, P.J., (1995). Principles of Haematology.1st edition. Wm. C. Brown Publishers.

6-Hanssler, L. and Roll, C. Increased thrombocyte count in newborn infants of drug dependent mothers. Clinical Pediatrics.1994, 206(1): 55-58.

7-Hiramatsu, M. Opium, Heroine and Morphine (A review). Nippon. Rishno.2010, 68(8):1506-1510.

8-Hsiao,G.; Shen,Y.; Fang,C.;Chou,D. and Lin,C. Morphine potentiated platelet aggregation in in vitro and platelet plug formation in in vivo experiments. J. Biomedical Science.2003, 10(3):292-301.

9-Jaimita, P.; Nagarathna, M. ; Rajani, B.; Pavni, M.; Madhu, B.; and Pravin, C. Role of oxidative stress and haeme oxygenase activity in morphine induced glomerular epithelial cell growth. American J. physiology.2003, 285:861-869.

10-Kapasi, A. A.; Coscia, S. A.; Pandya, M. P.and Singhal, P. C. Morphine modulates HIV-1$_*$gp160-induced murine

macrophage and human monocyte apoptosis by disparate ways. J. Neuroimmunol. 2004, 148,86-96.

11-Martin, W.R. and Fraser, H.F. A comparative study of physiological and subjective effects of heroin and morphine administered intravenously in postaddicts. J. Pharmacol. Exp. Ther. 1961, 133: 388–99.

12-Muslih, R.D.; Al-Nimer, O.M. and Al-Zamely, M.A. The level of Malondialdehyde after activation with (H2O2 and CuSO4) and inhibited by Desferoxamine and Molsidomine in the serum of patients with acute myocardial infection. Nat. J. Chem. 2002, 5:148-149.

13-Nagamatsu,K.; Ohno,Y. and Ikibichi,H. Morphine metabolism in isolated rat hepatocytes and its applications for hepatotoxicity. Biochemical Pharmacology. 1996, 35:3543-3548.

14-O'Neal, M.(2006). An Encyclopedia of Chemicals, Drugs, and Biologicals. Merck.

15-Patel, K.; Bhaskaran, M. ; Dani, D.; Reddy,K. and Szinghal, P. Role of haeme oxygenase (OH-1)in morphine – modulated apoptosis and migration of macrophage. J. Infection Dis.2003, 187:47-54.

16-Sharp, B. M.; Keane, W. F.; Suh, H. J.; Gekker, G.; Tsukayama, D. and Peterson, P. K. Opioid peptides stimulate superoxide production by human polymorphonuclear leukocytes and macrophages. Endocrinology . 1985, 117,793-795.29

17-Singhal, P. C.; Pamarthi, M.; Shah, R.; Chandra, D.and Gibbons, N. Morphine stimulates superoxide formation by glomerular mesangial cells. Inflammation . 1994, 18,293-299. 28

18-Weissman, D.E. and Haddox, J.D. "Opioid pseudoaddiction:an iatrogenic syndrome". Pain . 1989, 36 (3): 363–6.

19-Xu, Z.; Hou, B. ; Gao, Y. ; He, F. and Zhang, C. Effects of enriched environment on morphine-induced reward in mice. Exp. Neurol. 2007, 204 (2): 714–9.

20-Yunis, N. and Casson, I. An unusual cause of iron deficiency anemia in an intravienous drug users. Hospital Medicine. 2000, 61(1):62-63.