Hemodynamic, Thyroid and Immunomodulatory Effects of Heroin in Rats

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***Abstract*—Diacetylmorphine (heroin) has many effects on the body system; it exerts effects on cardiovascular, immune and endocrine systems. The aim of the this study is to investigate the short-term effects of low and high doses of heroin on systolic blood pressure (SBP), thyroid hormones and monocyte chemoattractant protein-1 (MCP-1). The experimental rats were divided into three groups, each with six individuals and the treatments were continued for seven days. SBP significantly reduced by heroin administration in the second dose as compared with the control group. A marked decrease in the serum NO level was also noticed after first (low) and second (high) dose of administration as compared with control group. The present results also revealed that serum MCP-1 was statistically increased in the second dose of heroin group. Statistical analysis showed that both serum T3 and T4 levels were reduced significantly by heroin administration. In conclusions, for the first time, our findings suggested that diacetylmorphine could affect immune system through MCP-1 elevation. As well as heroin may affect cardiac and liver functions via increasing troponin-T and bilirubin levels.**

***Index Terms*—Blood pressure, Heroin, MCP-1, Thyroxine, Troponin-T.**

I. Introduction

Heroin (diacetylmorphine) is an opioid analgesic synthesized adding two acetyl groups to morphine molecule. Morphine affects some physiological functions like hemodynamics, gastrointestinal function, respiration, and immunoregulation (Xu, et al., 1997), they also demonstrated that intravenous morphine enhances the NO levels through cholinergic and adrenergic mechanism. Furthermore, (Fonarow, 2002) resulted that may induce hypotension, bradycardia, regulation of sympathetic and parasympathetic nerve system through venodilation mechanism. Long term administration of morphine alters immune system (Szabo, et al., 1993) demonstrating that macrophages may be attenuate after morphine exposure. Strong evidence has been established in the modulation of the immune system by morphine administration. (Wetzel, et al., 2000) indicated that μ-opioid may change produce MCP-1, which has an important role in proinflammatory reactions and cell-mediated immune responses.

On the other hand, heroin may alter some endocrine glands. Recently, (Bhoir, et al., 2009) reported that heroin caused necrosis in the follicular epithelial cells of the thyroid gland. Furthermore, most recent data showed that addiction of opium could cause elevation of thyroid stimulating hormone (TSH) and reduction of T4 hormone. Besides those above effects of heroin, the wide spectrum of kidney alteration was found among heroin users (Cunningham, et al., 1984). Also, (Dettmeyer, et al., 2005) revealed that heroin is highly associated with nephropathy. The effects of diacetylmorphine on hemodynamics, immune system, and thyroid hormone are largely unknown. Accordingly, the aim of the current study was to investigate the short-term effects of a low and high dose of heroin on blood pressure, troponin-T (a marker for acute myocardial infarction), thyroid hormones and MCP-1 as a marker in proinflammatory reactions and cell-mediated immune responses.

II. Materials and Methods

A. Morphine (Heroin) Preparation

An adequate amount of diacetylmorphine or diamorphine or heroin was obtained by Narcotics Control Directorate in Erbil province-Iraq. For laboratory preparation, 250 mg of diamorphine powder was mixed with 25 mg of citric acid, then the mixture was dissolved in 0.8ml of D.W at 40 ^{o}C, they mixed well by using tip of needle, then the solution was heated over a flame until observing bubbles and the remaining solution was taken by 1ml then it diluted to 5mg/ml. One ml was injected to 1kg body weight (b.w.) of rats (5mg Heroine/kg b.w. rats), while another dilution was taken for the second dose by suspending the stock solution in sterilized distilled water.

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B. Animals and Housing

Eighteen adult female albino rats were involved in this study. All rats were weighing about (250 - 290 grams) at the time when the injection started. Rats were housed in standard plastic cages and they bedded with wooden chips. They were housed under normal laboratory conditions like 12:12 dark / light photoperiod at 23±2 ^{o}C. The rats were given standard pellets and tap water *ad libitum*.

C. Experimental Design

The current experiment was designed to study the impacts of two doses of heroin on blood pressure, thyroid and renal function measurements. The experimental rats were divided into three groups, each with six individuals and the treatment injections were continued for seven days as the following:
Group 1: Control. The animals were injected with normal saline and the rats were given standard rat chow. Group 2: Dose 1. The rats were given standard rat chow and they were injected with diacetylmorphine (1 mg/kg) intraperitoneal. Group 3: The rats were given standard rat chow and the animals were injected with diacetylmorphine (5 mg/kg) intraperitoneal.

D. Collection of Blood Samples

At the end of the experiment, the animals were anesthetized by 50 mg/kg of ketamine.

The sample of blood were taken by cardiac puncture into plastic tubes and centrifuged at 3000 rpm for 20 minutes. The sera were stored at –80 ^{0}C until use.

E. Blood Pressure and Heart Rate Measurements

Measurements of SBP and heart rate were measured by the tail-cuff method in all groups using power Lab. (AD Instruments, power lab 2/25). The rats were placed in a special rat restrain chamber and they warmed up to about 37 ^{o}C. Five readings were taken for each rat, and the highest and lowest values were neglected. The average was taken of the remaining readings.

F. Biochemical Determination

Serum Total Nitric Oxide Measurement

Serum total NO was determined by NO non –enzymatic assay kit (US Biological, USA).

Determination of Serum T_3, T_4 and Troponin-T

Serum T_3, T_4 and Troponin-T were determined by electrochemiluminescnce immunoassay "ECLIA" using Elecsy and Cobas immunoassay analyzers.

Determination of serum creatinine level

Creatinine level was determined by colorimetric method kit (BIOLABO. SA, France). Creatinine in alkaline picric acid solution, forms a color complex in which the absorbance was measured at 490 nm using spectrophotometer.

Determination of serum urea

Urea was determined by enzymatic test kit (BIOLABO. SA, France). The color intensity was measured at 600 nm.

Determination of serum total protein

Serum total protein was determined by biuret method, using colorimetric test kit (Biolab, France).

Determination of Serum Albumin

Serum albumin was determined by BCG method, using colorimetric test kit (Biolab, France).

Determination of Serum Total Bilirubin

Serum total bilirubin was determined by sulfanilic acid method ((BIOLABO. SA, France).

Determination of Serum Sodium, Potassium and Chloride Ion Concentrations

Serum Na^{+}, K^{+} and Cl^{-} ion concentrations were determined by using automated electrolyte analyzer (ELITE, USA).

Serum total calcium ion determination

Ca^{2+}-Kit enables colorimetric determination of total calcium without deproteinization. In serum, the calcium kit reacts with methylthymol blue indicator (MTB) in an alkaline medium. The color intensity of the Ca^{2+}-MTB complex, measured at 612 nm, is proportional to the quantity of calcium present in the sample. The kit was obtained from (BIOLABO.SA, France).

Determination of serum inorganic phosphate

Serum inorganic phosphate was determined by the ultra violet method. The absorbance measured at 340 nm is proportional to phosphate ions in the specimen (BIOLABO . SA, France).

G. Statistical Analysis

The obtained data were expressed as means $\pm$ standard error (SE) and statistical analysis was performed using statistical programmed for social science (SPSS version 15). Analysis of data was made by one-way analysis of variance (ANOVA). Then the comparisons between groups were done using Duncan post hoc analysis. P values <0.05 are considered as statistical significant.

III. Results

As shown in Fig. 1, SBP significantly reduced by heroin administration in the second dose (108.66 ± 4.200 mm Hg) as compared with the control group(123.66 ± 5.841 mmHg). On the other hand, H.R was lowered by heroin in the first dose of it administration (Fig. 2). A marked decrease in the serum NO level was also noticed after the first and second dose of administration as compared with control group (Fig. 3).

Table I shows that serum Na+ concentration tended to decrease significantly in the second dose while no significant differs were observed in serum PO-4, K+ and Cl- concentrations. Diacetylmorphine administration significantly decreased serum ionized Ca+2 from 1.270 ± 0.109 mmol/L in control rats to 0.876 ± 0.0242 mmol/L in heroin group.

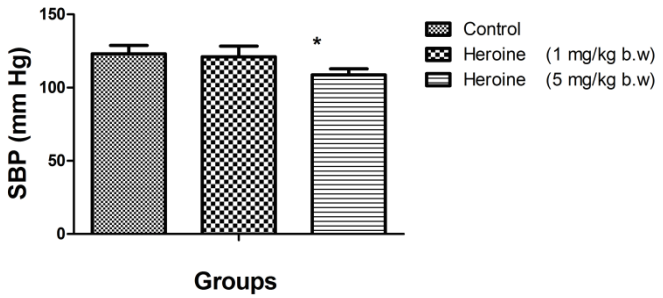

Fig. 1. Effects of heroin on SBP in rats.

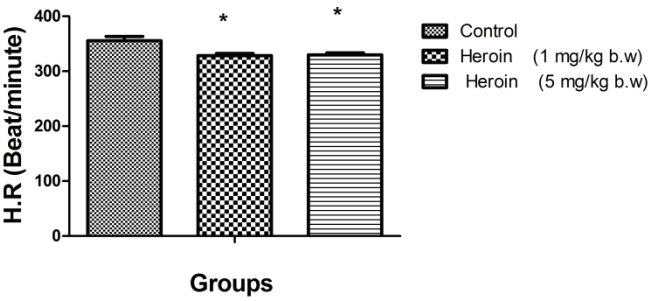

Fig. 2. Effects of heroin on H.R in rats.

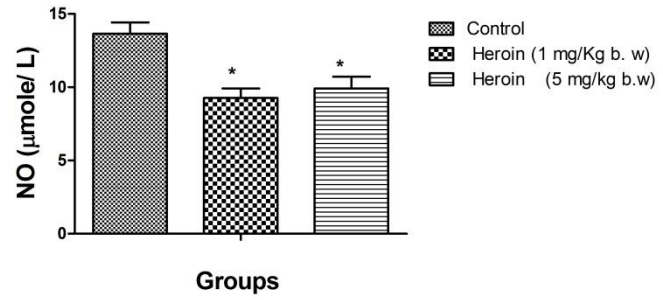

Fig. 3. Effects of heroin on serum NO in rats.

Table I shows that serum Na^+ concentration tended to decrease significantly in the second dose while no significant differs were observed in serum PO^-_4, K^+ and Cl^- concentrations. Diacetylmorphine administration significantly decreased serum ionized Ca^{+2} from 1.270 ± 0.109 mmol/L in control rats to 0.876 ± 0.0242 mmol/L in heroin group.

In Fig. 4, the results reveale that serum MCP-1 was statistically increased in the second dose of heroin group (22.70 ± 4.736 ng/ml) as compared with control rats (7.857 ± 1.208 ng/ml)

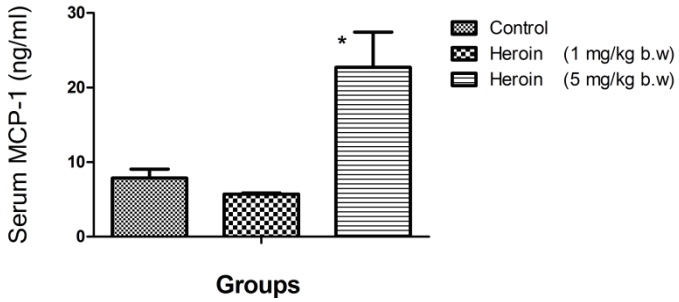

Fig. 4. Effects of heroin on serum MCP-1 in rats.

Statistical analysis also revealed both serum T_3 and T_4 levels was reduced significantly by heroin administration. Table II shows some liver and renal function test parameters; here only serum total bilirubin and serum urea were increased, whereas, serum total protein, serum albumin and serum creatinine did not statistically differ among the studied groups.

Serum troponin-T did not significantly alter in the first dose (Table II), while second dose caused a marked decrease in troponin-T concentration (0.3517 ± 0.0878) as compared with control group (1.652 ± 0.760).

TABLE I
EFFECTS OF HEROIN ON SERUM T_3, T_4, ELECTROLYTES, CALICUM AND PHOSPHATE IN ALBINO RATS.

Groups Parameter	Control	Heroin (1 mg/Kg b.w)	Heroin (5mg/Kg b.w)
T_3 (nmol/L)*	1.4360±0.0271[a]	1.3200±0.0255[ab]	1.0967±0.1330[b]
T_4 (nmol/L)*	55.798±5.0165[a]	54.768±3.5061[a]	38.455±4.8212[b]
Serum Na^+ *(meq/L)	167.40±2.2494[a]	164.40±2.2045[a]	142.20±6.7852[b]
Serum K^+*(meq/L)	4.9400±0.1326[a]	4.8200±0.1462[a]	4.6200±0.1496[a]
Serum Cl^- *(meq/L)	122.00±4.3703[ab]	125.20±1.5297[b]	110.60±5.3254[a]
Serum Ca^{+2} *(mg/dL)	1.2700±0.1090[a]	1.1800±0.0406[a]	0.8760±0.0242[b]
Serum PO_4^- *(mg/dL)	9.0930±1.4398[a]	9.5543±1.10899[a]	10.6008±1.1806[a]

TABLE II
EFFECTS OF HEROIN ON SERUM TROPONIN-T, SOME LIVER AND RENAL FUNCTION TEST PARAMETERS IN ALBINO RATS

Groups Parameter	Control	Heroin (1 mg/Kg b.w)	Heroin (5 mg/Kg b.w)
Serum troponin-T *	1.6520±0.7601[a]	1.8267±0.3414[ab]	0.3517±0.0878[b]
Serum total protein (gm/dL)	8.8000±0.4131[a]	8.9333±0.5529[a]	9.4000±0.2250[a]
Serum bilirubin (mg/dL)*	0.1345±0.0150[a]	0.1960±0.0066[b]	0.2713±0.0166[c]
Serum creatinine (mg/dL)	0.5351±0.080[a]	0.393±0.1139[a]	0.807±0.2733[a]
Serum urea (mg/dL)*	20.60±3.2749[a]	22.72±3.9487[a]	80.60±19.84[b]
Serum uric acid (mg/dL)*	13.055±1.0243[a]	11.111±0.7657[ab]	9.1667±1.4433[b]

The data is expressed as mean ± S.E. The same letters mean no significant differences. The different letters mean significant differences * P<0.05 and according to 1 way ANOVA followed by Duncan post hoc test.

IV. Discussion

As shown in Fig. 1, statistical analysis revealed that SBP was significantly decreased in heroin administered rats as compared with control. The possible mechanism of BP reduction would be through direct inhibition of the sympathetic nervous system (Fonarow, 2002; Mori, et al., 1998). On the other hand, Chang, et al. (2012) reported that diacetylmorphine users show decreased cardiac vagal activity. The reduction in heart rate (H.R) as obtained by the present result (Fig. 2) also may be involve in decreasing blood pressure (BP) which is consistent with (Newby, et al., 2007) showing that heroin administration is promptly induced marked bradycardia (and a concomitant reduction in cardiac output. Interestingly, the result of the present study illustrated in Table II, showed that heroin administration for seven days had a reduction effect on serum troponin-T. Liu, et al. (2011) resulted that heroin significantly can ameliorate hemodynamic parameters and reduce serum troponin I concentration. Calcium ions may also participate in such reduction of BP, as seen in Table I, heroin administration caused a significant decrease in serum ionized Ca+2. It has also been reported that serum

Ca+2 concentrations of diacetylmorphine addicts shows a significant decrease compared to that of the control group (Li, et al., 2011). In our finding, however, serum NO level was significantly reduced by heroin administration as compared with control group (Fig. 3). (Habibey, et al., 2010) also resulted that morphine significantly could decrease plasma NO levels in rats. Such results may be due to the compensatory mechanism for decreased BP. Also, (Rezazadeh, et al., 2014) resulted that morphine significantly attenuate systolic blood pressure, diastolic blood pressure, and mean arterial pressure in the 2K1C animals; they also showed that serum concentrations of nitric oxide were decreased. It has also been suggested that intravenous morphine increases release of NO from spinal cord by α - adrenergic and cholinergic mechanism (Xu, et al., 1997). Another possible mechanism for antihypertensive effects of heroin may be associated with sodium homeostasis, because there is now well established that sodium ions has a major role for depolarizing membranes, hence producing vasoconstriction, sympathetic activation and hypertension (Barrett, et al., 2010). Although, little is known about the effect of diacetylmorphine on serum sodium, but according to the present result shown in Table II, serum urea as indicator for renal functions was significantly elevated and this is may be due its enhancement of kidney injury (Dettmeyer, et al., 2005) reporting that heroin associated with nephropathy. Although, recent studies observed that heroin significantly increase creatinine clearance (Javadian, et al., 2013). The present results also showed that diacetylmorphine could affect liver tissues. It markedly elevated serum total bilirubin (Table II). Researches on the effects of diacetylmorphine in renal diseases are also very limited. Morphine-induced renal function in terms of serum urea and creatinine level was reported by (Sumathi, Niranjali and Devaraj, 2009).

Interestingly, and for the first time, we found that diacetylmorphine administration caused a significant elevation in serum MCP-1 levels. It is a potent chemoattractant, which has ability to promote monocyte recruitment as well as activates macrophages and monocytes. (Fuentes, et al., 1995). However little are known about the relation of diacetylmorphine and immunomodulation, although the present finding of MCP-1 elevation (Fig. 4) supports this modulation of immunity. According to our knowledge, this is the first study shows the beneficial effects of diacetylmorphine in immunomodulation through MCP-1 elevation. However, it has been postulated that μ-Opioids are capable of altering inflammatory response and the release of cytokines (Wetzel, et al., 2000). Also (Hatsukari, et al., 2005) reported that morphine-induced modulation of macrophage acts as immunosuppressive due to the requirement of macrophage recruitment. As shown in Table I, heroin administered rats reduced serum T3 and T4 significantly. The rational reason for these reductions may be through thyroids stimulating hormone (TSH) inhibition (Moshtaghi-Kashanian, et al., 2005). However, further studies need to explain the exact mechanism by which heroin reduces thyroid hormones.

V. Conclusion

The results suggested that heroin may have beneficial effects in modulating BP, thyroid hormones and immune system, but it also alters liver and kidney functions, the results also suggest that the hypotensive effects of heroin may be returned to its nitric oxide and potassium modulation. For the first time, the present results recorded that heroin could affect immune system through macrophage stimulation to release MCP-1 into the blood. As well as heroin affects cardiac and liver functions via increasing troponin-T and bilirubin levels.

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