

Changes in the hemodynamic and thyroid functions of rats treated by opium

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Abstract

Opioids are perhaps the most efficacious analgesic agents influencing a large number of body functions. The objective of this study is to observe changes in hemodynamic, electrolytes, and kidney and thyroid functions of opium treatment in rats. Thirty male rats were randomly distributed into three groups. Group 1 regarded as control, while in group 2 and 3, the animals were daily injected intraperitoneally with opium for seven successive days. Intraperitoneal opium injection caused a dose-dependent increase in serum calcium (Ca^{+2}) and phosphate (PO_4^-) levels, whereas the nitric oxide (NO), triiodothyronine (T_3) and thyroxine (T_4) levels were significantly decreased in both doses. The significant decrease in serum (NO) level and increase serum (Ca^{2+}) and (PO_4^-) levels resulted in significant elevation of systolic blood pressure (SBP) accompanied by elevation of serum bilirubin and urea. Our finding suggests that opium causes hypertension, kidney and thyroid function abnormalities mostly in concentration dependent manner.

Keywords: Opium, hemodynamic, nitric oxide, T_3 and T_4 .

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التغيرات في الدورة الدموية و وظيفة الغدة الدرقية لدى الجرذان المعالجة بالأفيون

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المخلص

تعد المواد الأفيونية من المسكنات الأكثر فاعلية عن طريق تأثيرها على عدد كبير من وظائف الجسم. الهدف من هذه الدراسة هو المراقبة التغيرات في الدورة الدموية، الاملاح المعدنية، والكلية والغدة الدرقية بعد حقن الجرذان بالأفيون، تم توزيع ثلاثين ذكور من الجرذان عشوائيا إلى ثلاث مجموعات. المجموعة الأولى اعتبرت السيطرة، بينما في المجموعة الثانية والثالثة حقن الحيوانات بالأفيون عن طريق البريتوني لمدة سبعة أيام متتالية. تسبب حقن الأفيون زيادة في الكالسيوم ومستويات الفوسفات متناسبة مع الجرعات، في حين أن مستويات اكسيد النترريك ثلاثي يودثريونين، وهورمون الغدة الدرقية انخفضت بشكل ملحوظ في كلا الجرعتين . وادت انخفاض كبير في المصل النترريك الأوكسيد وزيادة مستوى الكالسيوم والفوسفات في ارتفاع كبير في ضغط الدم الانقباضي يرافقه ارتفاع البيلبيريوبين واليوريا في الدم. وتشير نتائج البحث بأن الأفيون قد يسبب ارتفاع ضغط الدم والكلية و وظيفة الغدة الدرقية وغالبا ما يزداد مع زيادة الجرعات.

كلمات البحث: الأفيون، الدورة الدموية، اوكسيد النترريك T_3 ، T_4 Introduction

The word of opium is coming from the Greek name for juice, the drug is obtained from the juice of the poppy *Papaver somniferum* [1]. Historically, it has been indicated that opium was used in the Middle East as early as 4,000 BC. Later, the Greeks and Romans fully aware of the analgesic and euphoric properties of opium used as a drug for pain relief and recreation [2]. It has been found that half of the world's opioid users (7.8 million) are Asian with the highest prevalence rates reported from Iran, Kyrgyzstan and Laos [3]. Commercially, opium is utilized

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as the raw material for the production of some drugs such as morphine, codeine, papaverine and noscapine which constitute 8-17%, 1-10%, 0.5-1.5% and 0.7-5% of opium, respectively [4]. On the other hand, a wide range of peptides that possess opioid-like actions have been identified in the central nervous system and peripheral tissues, such as endorphins, enkephalins, endomorphins, and dynorphins [2]. It is well known that endogenous opioid peptides as well as exogenously administered opioids exert their effects through the opioid receptors that have a high degree of structural similarity in the transmembrane and intracellular domains [5]. Recently, the researchers identified four various opioid receptor systems, including Mu (μ), Delta (δ), Kappa (κ), opioid receptor like-1 (ORL1). The genes of these opioid receptor systems have been characterized at molecular, cellular, and pharmacological levels [6]. Most of the studies have shown that the effects of opium are mostly from its morphine which suppresses the active reaction to pain impulses especially in the spinal cord and through modulation of central pain processing [4]. However, long-term use of this drug can be connected with some pathological consequences including neurotoxicity and neural dysfunction, respiratory disorder [9] oxidative stress and apoptosis [8]. Moreover, Kouros *et al.*, [2010] indicated that opioid exposure can cause alterations in both the physiology and structure of the kidney [9]. Several investigations about the effects of opium on the cardiovascular system have been performed; they show that hypertension and bradycardia, are among the cardiovascular effects of opium [10]. May *et al.*, [1989] investigated that intravenously given morphine caused a centrally mediated increase in sympathoadrenal outflow and increase blood pressure in conscious rabbits [11]. Most of the studies have shown that the effects of opium are mostly from its morphine which suppresses the active reaction to pain impulses especially in the spinal cord and through modulation of central pain processing [4]. However, long-term use of this drug can be connected with some pathological consequences including neurotoxicity and neural dysfunction, respiratory disorder [9] oxidative stress and apoptosis [8]. Moreover, Kouros *et al.*, [2010] indicated that opioid exposure can cause alterations in both the physiology and structure of the kidney [9]. Several investigations about the effects of opium on the cardiovascular system have been performed; they show that hypertension and bradycardia, are among the cardiovascular effects of opium [10]. May *et al.*, [1989] investigated that

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intravenously given morphine caused a centrally mediated increase in sympathoadrenal outflow and increase blood pressure in conscious rabbits [11].

Materials and methods**Animals**

Thirty female albino rats *Rattus norvegicus* weighting about (200-250) grams were bred in the animal house of Biology Dept. /College of Science/Salahaddin University –Erbil.

Drug Preparation

The extract of Opium was obtained from the Directorate of Narcotics Control in Erbil province/Iraq. The stock solution of Opium was prepared by dissolving 2.5gms of opium extract in 50 ml of 35% diluted ethanol creating a concentration of (50mg/ml). The amount of 1ml of the prepared solution was injected intraperitoneally per 1kg of b.w (50mg/kg). Further dilution was made for preparation of 25mg/kg rat b.w [18].

Experimental Design

The rats of each experiment were divided into three groups:

Control group (6 rats): The rats of this group were fed with standard rat diet without any treatments. Opium 1st dose (6 rats): The rats of this group were fed with standard rat diet and treated with intraperitoneal injection of 25mg/kg/day opium for a week. Opium 2nd dose (6 rats): The rats of this group were fed with standard rat diet and treated with intraperitoneal injection of 50mg/kg/day opium for a week.

Collection of blood samples

At the end of the week of treatment, the rats were anesthetized using ketamine hydrochloride (50 mg/kg) and xylazine (20). Blood samples were drawn by cardiac puncture and the tubes were centrifuged at 3000 rpm for 20 minutes; then, the sera samples were stored at -85C° until performing the assays.

Blood pressure and heart rate measurements

Systolic blood pressure and heart rate were measured by the tail-cuff method in all groups using power Lab (AD Instruments, power lab 2/25). Rats were placed in a restraining chamber and

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warmed to an ambient temperature of about 37°C, for 10-15 minute, then occluding cuffs, and pneumatic pulse transducers were placed on the rats' tails. Five readings were taken for each animal, the highest, lowest and any associated with excess noise or animal movement were neglected.

Biochemical determination**Serum total nitric oxide measurement**

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

Determination of serum T₃ and T₄: Serum T₃ and T₄ were determined by electrochemiluminescence immunoassay “ECLIA” using Elecsy and cobas immunoassay analyzers.

Determination of serum sodium, potassium and chloride ion concentrations:

Serum levels of Na⁺, K⁺ and Cl⁻ were determined by electrolyte analyzer (ELITE), electra biomedical corporation (USA).

Serum total calcium ion determination: Colorimetric Ca²⁺-Kit (BIOLABO.SA, France) was used for determination of total calcium, without deproteinization. The color intensity of the Ca²⁺-MTB complex is proportional to the amount of calcium in the sample.

Determination of serum creatinine level: The level of creatinine was determined by colorimetric method kit (BIOLABO. SA, France). Creatinine forms a color complex substance in alkaline picric acid solution. The absorbance of it was measured at 490 nm.

Determination of plasma 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)

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

Statistical analysis

All statistical data were presented as means \pm standard error (SE) and statistical analysis was performed using available statistical software (SPSS version 15). One-way analysis of variance (ANOVA) was used for data analysis. Duncan's test was carried out for post hoc analysis. P values <0.05 were considered as significant.

Results

Administration low dose of opium (25 mg/kg b.w) increased blood pressure significantly (129.3 ± 2.5776 mmHg) versus control rats (109.3 ± 1.940 mmHg) within a week of treatment. While a greater elevation in systolic blood pressure (145.5 ± 7.1355 mmHg) was recorded when the rats supplemented with high dose (50 mg/kg diet) of opium within the same period of treatment, as compared with control rats (Figure 1, A). Statistical analysis showed no significant differences between animals supplied with different doses of opium in heart rate (Figure 1, B).

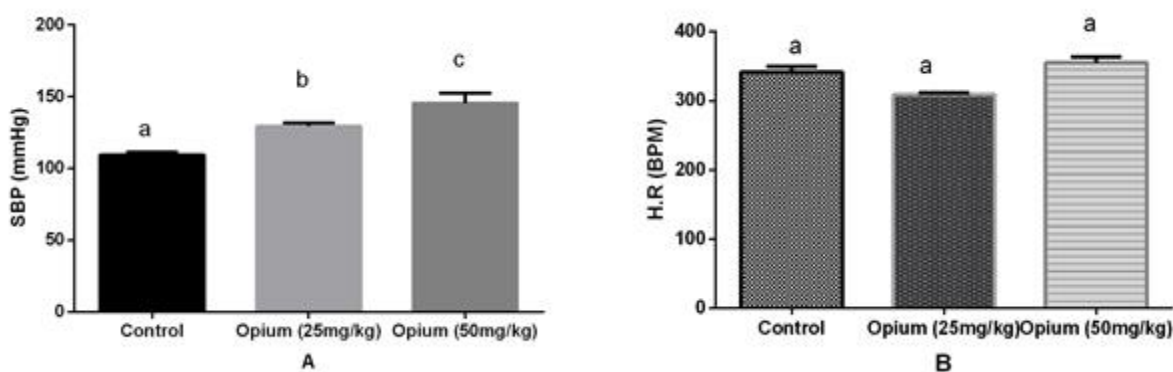


Figure 1: (A) Elevation of SBP in a concentration-dependent manner by using two doses of opium. (B) No significant change in HR in different doses of opium (25 and 50 mg/kg). The different letters on bars mean significant and the same letters on bars mean no significant difference. The data represented mean \pm SEM *P <0.05 considered a significant difference according to 1-way ANOVA followed by Duncan post hoc test.

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Serum total NO level decreased in both low and high opium dose treated rats ($11.12 \pm 1.0670 \mu\text{mol/L}$ and $10.44 \pm 1.2541 \mu\text{mol/L}$) respectively, compared to control group ($15.34 \pm 1.940 \mu\text{mol/L}$) (Figure 2). In animals treated with low opium dose, Ca^{2+} concentration tended to be higher than in control group, but the differences were not significant. Serum Ca^{2+} level was significantly ($p < 0.05$) increased in high opium dose treated rat group as compared with control group (Table 1). Serum PO_4^- level significantly ($p < 0.01$) increased only when rats treated with high opium dose (50 mg/kg b.w) when compared with control group (Table 1). On the other hand, statistical analysis shows no significant differences between animals supplied with different doses of opium in heart rate and serum electrolytes (Na^+ , K^+ and Cl^-) levels (Table 1).

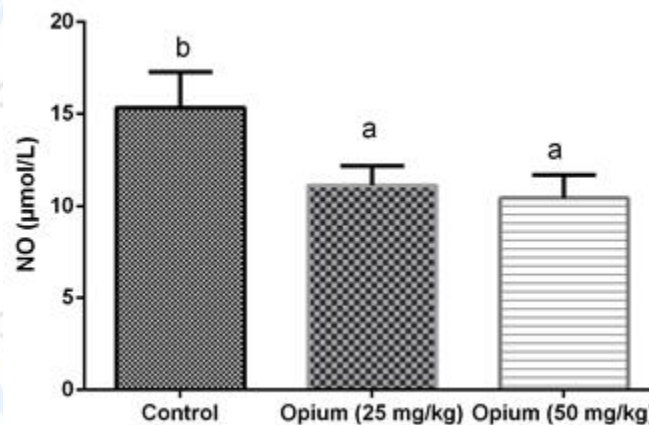


Figure (2): Nitric oxide level in different doses of opium (25 and 50 mg/kg) treatment. Opium treated rats were compared with control rats. The different letters on bars mean significant and the same letters on bars mean no significant difference. The data represented mean \pm SEM * $P < 0.05$ considered a significant difference according to 1-way ANOVA followed by Duncan post hoc test.

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Table (1): Effects of opium on serum Na⁺, K⁺, Cl⁻, Ca²⁺ and PO₄⁻.

Groups Parameters	Control	Opium(25mg/Kg b.w)	Opium(50mg/Kg b.w)
Serum Na ⁺	159.3±8.7737 ^a	150.3±5.4994 ^a	159.3±7.5571 ^a
Serum K ⁺	5.1000±0.1653 ^a	4.7333±0.1706 ^a	4.7667±0.1382 ^a
Serum Cl ⁻	119.0±6.0166 ^a	113.3±3.9972 ^a	120.8±5.8046 ^a
Serum Ca ⁺⁺ *	9.619±0.3012 ^a	10.52±0.3411 ^{ab}	10.78±0.3849 ^b
Serum PO ₄ ⁻ *	6.4535±0.8849 ^a	5.9302±9381 ^a	10.2558±1.4380 ^b

The data represented mean ± SEM *P<0.05 considered a significant difference according to 1-way ANOVA followed by Duncan post hoc test.

Serum T₃ concentrations were significantly (p<0.05) decreased in both opium group without significant difference between them when compared with control group (1.5683±0.7391). Low opium dose group (1.3860±0.03736) and high dose group (1.2700±0.0328) (Figure 3, A). Intraperitoneal opium injection significantly (p<0.05) decreased T₄ level in a dose- dependent manner. In high dose group, serum T₄ level was significantly (p<0.05) lower (26.87± 1.5031) than low dose group (41.00±4.0794), versus control group (58.19±5.5021) (Figure 3, B).

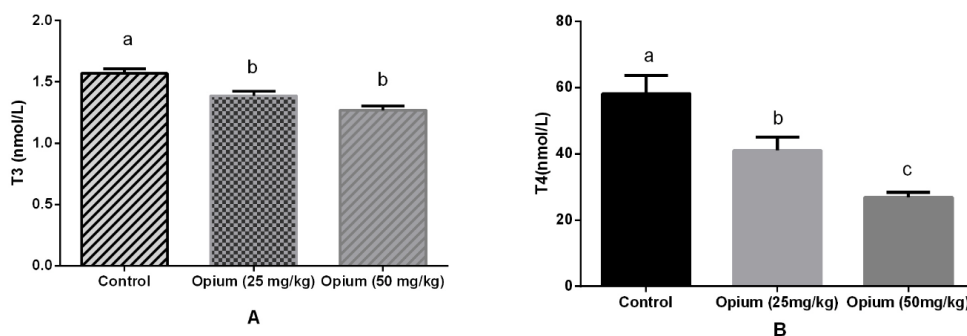


Figure (3): (A) Reduction of T₃ hormone levels in different doses of opium (25 and 50 mg\kg) treatment. (B) Reduction of T₄ in a concentration dependent manner by using two doses of opium. The different letters on bars mean significant and the same letters on bars mean no significant difference. The data represented mean ± SEM *P<0.05 considered a significant difference according to 1-way ANOVA followed by Duncan post hoc test.

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In animals treated with low opium dose, serum total bilirubin concentration tended to be higher than in control group but the difference was not significant (0.1824 ± 0.02102). Serum bilirubin level was significantly ($p < 0.05$) increased in high opium dose treated rat group (0.2918 ± 0.05672) as compared with control group (0.1140 ± 0.01571) (Figure 4).

Oral administration of two doses of opium (25 and 50 mg/kg b.w) failed to change serum total protein, albumin (Table 2).

Serum urea did not change significantly in the rats treated with low dose of opium compared with control group. In contrast higher opium dose dramatically increased urea level in comparison to control group (Table 2). Dietary opium content in different doses did not make significant change in creatinine and uric acid level in any of two dietary opioids treated groups (Table 2)

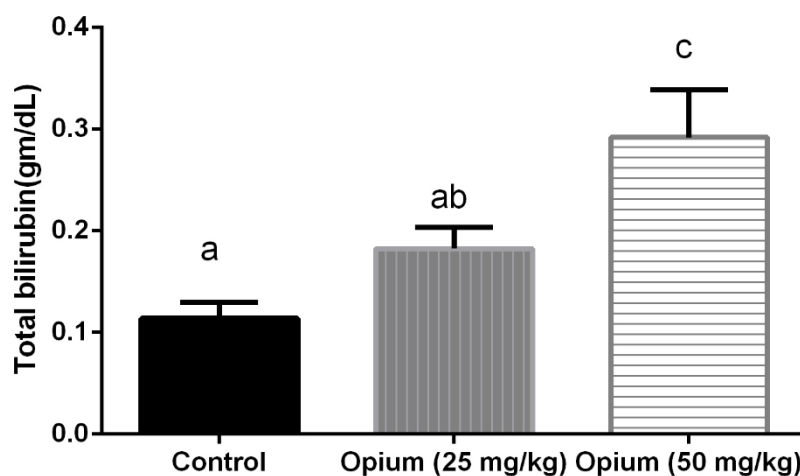


Figure (4): Serum total bilirubin concentration in different doses of opium (25 and 50 mg/kg) treatment. Opium treated rats were compared with control rats. The different letters on bars mean significant and the same letters on bars mean no significant difference. The data represented mean \pm SEM * $P < 0.05$ considered a significant difference according to 1-way ANOVA followed by Duncan post hoc test.

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Table (2): Effects of opium on serum total protein, albumin, creatinine and urea.

Groups Parameters	Control	Opium(25mg/Kg b.w)	Opium(50mg/Kg b.w)
Serum total protein (gm/dL)	7.2000±0.4953 ^a	7.2000±0.2065 ^a	6.8667±0.4779 ^a
Serum albumin (gm/dL)	2.4082±0.2133 ^a	2.8207±0.0779 ^a	2.9357±0.2272 ^a
Serum creatinine(mg/dL)	1.0855±0.0799 ^a	1.1197±0.1970 ^a	0.9829±0.2099 ^a
Serum urea (mg/dL)	22.4215± 3.23368 ^a	25.1121±4.70033 ^a	45.4410±5.87046 ^b
Serum uric acid (mg/dL)	9.9605± 0.65998 ^a	9.7588± 0.92131 ^a	10.8553± 0.87856 ^a

The data represented mean ± SEM *P<0.05 considered a significant difference according to 1-way ANOVA followed by Duncan post hoc test.

Discussion

The current results revealed that opium administration in rats leads to the alteration in several variables associated with cardiovascular, hemodynamic and thyroid functions. Several mechanisms could be proposed for explaining the hypertensive action of opium administration. It could be related to the change in NO production, since we found significantly lower NO levels in opium treated rats. It has been also reported that opium inhibits NO production [21], which in turn leads to vasoconstriction and hypertension [22]. On the other hand, the increasing BP following opium treatment may be due to elevation in serum total calcium, which is known to increase blood pressure [23]. The other reason which can explain the onset of hypertension in our study is the elevation in serum phosphate level. It was reported that opium drug can lead to hyperphosphatemia [24], which leads to mineral deposition in the vascular wall and arterial wall stiffness [25], so it can be considered as a factor for incidence of hypertension. Moreover, the results demonstrated that the elevated blood pressure may not be associated with electrolyte changes since opium did not change serum electrolytes, Na⁺, K⁺ and Cl⁻. It has been indicated that opium stimulates sympathoadrenal outflow which increases plasma catecholamines and

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causes alpha-adrenoreceptor-mediated vasoconstriction [11]. Another possible mechanism for hypertensive effects of opium is that opium increases plasma vasopressin concentration and consequently elevates blood pressure [26].

The results of the present investigation showed that opium decreased serum NO in a dose-dependent manner. The inhibitory action of opium on NO reported to be through modulation of cGMP and/or other signaling molecules [13]. While the direct interaction of opium with nitric oxide synthase (NOS) enzyme activity resulted in a reduction of it [27]. However, Lyse *et al.*, [2001] revealed that metabolite of morphine, morphine-6 beta-glucuronide, induces a pronounced reduction of inducible nitric oxide synthase (iNOS) and it also produces a reduction in the level of plasma nitrite/nitrate, the more stable end product of NO degradation [28].

In our study, opium caused a significant increase in serum Ca^{+2} level. Studies indicated that opioid drugs can lead to respiratory depression and acidemia [7]. Aguiler *et al.*, [2000] postulated that acidemia decreases the binding of calcium to plasma protein with subsequent increases in the ionized calcium [23]. The current results revealed a significant increase in serum bilirubin in opium treated rats. Studies reported that presence of elevated serum concentration of bilirubin is a principal sign of cholestasis [29]. However, Hashemzahi M *et al.*, [2008] that narcotic opiate addiction is a major risk factor for the occurrence of acute cholecystitis [30]. On the other hand, plasma bilirubin concentration increased in the patients with opium-induced biliary cirrhosis, and it suggested that there may be a metabolic contribution to the cholestasis of primary biliary cirrhosis that is caused by increased activity by the opioid peptide [31]. The treatment of a high dose of opium markedly increased the level of serum urea, the end product of protein catabolism, [32] which may be related to the opium's ability to stimulate cortisol secretion by inner zona glomerulosa cells [33] Since Atlan *et al.*, [2010] mentioned that elevation of cortisol level leads to increase in catabolism rate of proteins to generate glucose in gluconeogenesis process [34]. Furthermore, acute renal diseases that affect drug users include myoglobinuria and glomerulonephritis, with elevated levels of serum myoglobin associated with an elevation in blood urea [35].

The current results showed that opium treated rats have a lower serum level of total T_3 and T_4 compared to control rats. In a study performed on ten heroin-dependent men, no difference was

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observed between treated and control groups regarding TSH, T₃ and T₄ serum levels [36]. But in another study performed in Iran, opium consumption has been associated with an increase of total T₃, T₄ and TSH [37]. The difference between the results of mentioned studies and those of the current study might be due to opium various amounts of alkaloid composition [38], which are about 70 ingredients [39]. So their impacts can be vary in comparison pure morphine, noscapine, papaverine and heroin. The reasons for lowering serum T₃ and T₄ in our study may be due to the oxidative action of opium as there is a growing body of evidence indicating that morphine can elicit oxidative stress [8]. Though, oxidative stress plays a significant role in the development of hypothyroidism [40]. However, prolonged stress may cause an elevation in cortisol concentrations as the adrenal glands respond to the stress. The high cortisol levels inhibit the conversion of T₄ to T₃ [41]. On the other hand, loss of thyroid-binding protein in urine under the effects of oxidative stress could be another cause of decrease in serum T₃ and T₄ levels.

Conclusions

From these findings we can relate the elevation of blood pressure in opium rats to the reduction of NO levels and elevation of serum calcium concentrations. In addition, alteration in thyroid and kidney functions in a dose-dependent manner.

References

1. Goodman GA, Gooman LS: The pharmacological basis of therapeutics. 7thed. McGraw Hill, New York 2001.
2. Holzer P: Pharmacology of Opioids and Their Effects on Gastrointestinal Function. Am J Gastroenterol Suppl 2014, 2:9– 16.
3. Joukar S, Najafipour H, Malekpour-Afshar R, Mirzaeipour F, Nasri HR: The effect of passive opium smoking on cardiovascular indices of rabbits with normal and ischemic hearts. Open cardiovascular MedJ 2010, 4:1-6.
4. Haghpanah T, Afarinesh M, Divsalar K.: A Review on hematological factors in opioid-dependent people (opium and heroin) after the withdrawal period. Addiction and health 2010, 2(1-2): 9–16.

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5. McNally GP, Akil H, Davis KL, Charney D, COyle JT, Nemeroff C: Opioid peptides and their receptors: Overview and function in pain modulation. *Neuropsychopharmacology: 5th ed. Generation of Progress*. Lippincott Williams & Wilkins, 2002.
6. Al-Hasani R, Bruchas M,R: Molecular Mechanisms of Opioid Receptor-Dependent Signaling and Behavior. *Anesthesiology* 2011, 115(6): 1363–1381.
7. Dahan A, Aarts L, Smith TW: Incidence, reversal, and prevention of opioid-induced respiratory depression. *Anesthesiology* 2010, Vol.112, 226-238.
8. Skrabalova J, Drastichova Z, Novotny J: Morphine as a Potential Oxidative Stress-Causing Agent. *Mini Rev Org Chem* 2013, 10(4):367-372.
9. Kouros D, Tahereh H, Mohammadreza A: Opium and heroin alter biochemical parameters of human's serum. *The American journal of drug and alcohol abus* 2010, 36 (3) 135-139.
10. Shirani S, Shakiba M, Soleymanzadeh M, Esfandbod M: Can opium abuse be a risk factor for carotid stenosis in patients who are candidates for coronary artery bypass grafting? *Cardiology journal* 2009, 17(3): 254-258.
11. May CN, Whitehead Cj, DashwoodMr, MathiasCj: Investigation of the central sites at which morphine acts to cause hypertension in conscious rabbits. *Br. J. Pharmacol* 1989 (97), 873-881.
12. Lomniczi A, Mastronardi CA, Faletti AG, Seilicovich A, De Laurentiis A, McCann SM, Rettori V: Inhibitory pathways and the inhibition of luteinizing hormone-releasing hormone release by alcohol. *Proc. Natl. Acad. Sci. USA* 2009, 7: 2337 – 2342.
13. Bhargava HN, Bian JT and Kumar S: Mechanism of attenuation of morphine antinociception by chronic treatment with L-arginine. *J. Pharmacol* 1997, 281: 707 – 712.
14. Toda N, Kishioka S, Hatano Y, Toda, H: Modulation of Opioid Actions by Nitric Oxide Signaling. *Anesthesiology* 2009, 110:166–81

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15. Benyo Z, Szabo C, Velkei MH, Bohus B, Wahl M, Sandor P: Intravenous beta-endorphin administration fails to alter hypothalamic blood flow in rats expressing normal or reduced nitric oxide synthase activity. *Peptides* 1996 17: 733 - 736
16. Mahani SE, Motamedi F, Ahmadiani A: Involvement of hypothalamic pituitary adrenal axis on the nifedipine-induced anti nociception and tolerance in rats. *Pharmacol Biochem Behav* 2006; 85:422–427.
17. Ipp E, Schusdzarra V, Harris V, Unger RH: Morphine-Induce Hyperglycemia: Role of Insulin and Glucagon. *Endocrinology* 2010.107(2):461-3
18. Gozashti MH, Mohammadzadeh E, DivsalarK, Shokoohi M: The effect of opium addiction on thyroid function tests. *Journal of Diabetes & Metabolic Disorders*. 2014, 13:5.
19. Vuong C, Van Uum SHM, O'Dell LE, Lutfy K, Friedman TC: opioids and opioid analogs on animal and human endocrine systems. *Endocrine Reviews* 2010, 31(1): 98-132.
20. Laird K, Swindle M, Fleckneell P: *Rodent and rabbit medicine*. First edition. BPC wheatons Ltd, Exeter U.K 1996.
21. Omer M, Sipahi EY, Umer H, Isikdemir F, Numnogl G, Barut F, Altunkaya H, Ozer Y, Ayoglu FN, Sipahi TH, Tekin IO, Banglu N: Morphine modulates inducible nitric oxide synthase expression and reduces pulmonary oedema induced by α -naphthylthiourea. *European journal of pharmacology* 2005, 511, 183-189.
22. Shepherd JT: Increased systemic vascular resistance and primary hypertension: the expanding complexity. *J Hypertens* 1990, 8 (7): S15–S27.
23. Aguilier M, Vaughan R.S: Calcium and the anaesthetist. *Anaesthesia* 2000, 55, 779±790.
24. Opioid-induced secondary adrenal insufficiency presenting as hypercalcaemia. *Endocrinal diabetes metab case rep* 2015,10.1530/EDM-15-0035.
25. Huang CX, Plantinga LC, Fink NE, Melamed ML, Coresh J, Powe NR: Phosphate Levels and Blood Pressure in Incident Hemodialysis Patients: A Longitudinal Study *Adv Chronic Kidney Dis*. 2008, 15(3): 321–331.

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26. Aziz LA, Forsling ML, Woolf CJ: The effect of intracerebroventricular injections of morphine on vasopressin release in the rat. *Journal of Physiology (London)* 1981, 311, 401-409.
27. Kampa M, Hatzogloul A, Notas G, Niniraki, Kouroumalis E, Castanas E: Opioids are non-competitive inhibitors of nitric oxide synthase in T47D human breast cancer cells *Cell Death and Differentiation* 2001, 8, 943- 952
28. Lyse DT, Carrige KA: Morphine induces-6- β -glucuronide modulates the expression of inducible nitric oxide synthase. *Inflammation* 2001, 25(4), 267-275.
29. Nathan Cherny, Marie F, Stein K, Russell P, David CC. *Oxford text book of palliative medicine*. Fifth edition. Oxford university press.
30. Hashemzahi M, Esmaili-Motlagh M, Moodi M, Balali-Mood M: Narcotic drug abuse and other risk factors in 100 operated patients for acute cholecystitis in Birjand, Iran. *Saudi Med J* 2008 May;29 (5):698-702.
31. Thornton JR, Losowsky M S: Opioid peptides and primary biliary cirrhosis. *BMJ*. 1988, 297 10 1501-1504.
32. Sylvia S, Mader W: *Biology*. 6th ed, McGraw, New York, 1998 p 185.
33. Gilles R, Heijer M, Rose AH, Sweep FCGJ, Wetzel JFM: Thyroid function in patient with proteinuria. *Netherl and journal of medicine* 2008, vol. 66 no.11. 483-485.
34. Atlan O, Popova-Ralcheva S, Moneva P, Aleksiv Y, Peeva T, Ilieva Y, Penchev P: Effect of heat stress on some physiological and biochemical parameters in buffaloes. *Italian J. Anim. Sci* 2010, 6(2): 1325-1328.
35. Eric C, Strain MD, Maxine LS: *The treatment of opioid dependence*. The Johns Hopkins university press. 2005.
36. Brambilla F, Nobile P, Zanoboni A, Zanoboni-Musiaccia W, Meroni PL: Effects of chronic heroin addiction on pituitary-thyroid function in man. *J Endocrinol Invest* 1980, 3(3):251-255.
37. Ghanei A, Banaei-Broujeni S, Malekzadeh G, Alvijeh MM, Zarepur E: Abstaining Opioid Drugs as a Possible Risk Factor for Graves' Disease. *Addiction Research & Therapy* 2012, vol 3

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38. Venturella VS, Gennard AR: The Science and Practice of Pharmacy. 19th ed. New York: Mack Publishing Company; 1995. 400- 402.
39. Buchbauer G, Nikiforov A, Remberg B: Constituents of opium. *Planta Med* 1994; 60(2): 181-3.
40. Ali WJH, Ali RKH, Alkafaluji S: The correlation between oxidative stress and thyroid hormones in serum and tissue homogenized of hypothyroidism patients. *Medical journal of Babylon* 2012, Vol 9-no 4. 834-849
41. Fernandez V, Castillo I, Tapia G, Romanque p, Uribe-echevarria S, Uribe M: Thyroid hormone. *Hepatology* 2007, 45(1): 170-177.

