Histophysiological Considerations Deals Damaging of Rat's Kidney by Examined Doses of Heroin

Abdul-Samad Uleiwi Hassan[1], Shatha Atta Abeed[2], Ashwaq Kadhem Obeid[3]

[1]Assit. Prof. Dr. Al-Forat Al-Awsat University, Health&Medical Techniques College, Clinical Pathology Department/ Iraq, [2]Assit. Prof. Al-Forat Al-Awsat Technical University/ Kufa Technical Institution, Animal Production Department/Iraq, [3]Assit. Prof. Dr. Kerbala University, Education for pure science College, Biology Department/Iraq

Abstract

Scientific gain after treating about 100lab rats with heroin of several doses to examine the quantitative physical, clinical chemistry and histopathological tests. Accompanied bymodern statistical analysis, the results explain an obvious differentiation ($p<0.05$) for urine creatinine, serum creatinine, creatinine clearance, 24-hour proteinuria, protein/creatinine ratio and urea; at the same time as other parameters showed slight and concealed splendour achievements results recorded ups or downs reported below for Weight, food intake, water intake and urine volume.

Judgment based on reasoning were proving the negative effects of heroin on the kidneys of the lab rats successes an acute damages on the histopathological levels.

Keywords: *Histophysiology, Heroin, Laboratory rats, Kidney's damaging.*

Introduction

Heroin (or *smack*, *horse*, *hell dust,* and *big H*) considered as an opioid compound gained from native material extracted from the seed of different opium poppy plants, that is morphine. Many countries planted heroin like Colombia, Afghanistan and Mexico. Heroin was be brown or white whey, or as black sticky/tarmaterial(1).

Heroin reacts with the brain swiftly through binding special receptors on the cells of many areas those encompassed in emotions of pleasure, pain, sleeping, ruling heart beatsandrespiration(2).

Misuse of some opioid pain pharmaceutics such as Vicodin® and OxyContin®looked to have belongings like that of heroin to an extents switches to using this drug in an estimated levels of 50 to 80 percent(3,4).Recent scientific researches suggests that heroin is oftentimes the top opioid used by one-third people whom recorded heroin as the premium opioid they were employed frequently to be passionate(5).

Aimof this project is to explain the alternative hypothesis for the correlation of heroin to the cases of acute kidney damage.

Materials and Methods

100 rat of lab model *Rattusnorvegicusdomestica* type Sprague-Dawley were beenbought from Hilltop lab animals, Inc. California. Those cabinet formed of 50 males vs 50 females. Body weight of animals was measured.

Heroin extracted in I.R. of Iran and it was injected via Intra-Peritoneal (IP) manner. Later animals were put in five bands:

1- Standard (n=20) were put in standard cage conditions.

2- Control (n=20) did not driven anyheroin transaction, just given 250 µL physiological saline periodically.

3- Test one (n=20) driven by physiological

saline and citric acid injected in a term of 33 days with the following order: from day 1-3 injection applied 3 times per day; in the first one given a dosage of 200µLphysiological saline plus 0.5µLcitric acid; 250µLphysiological saline plus 0.55µLcitric acid in the next day and 300µLphysiological saline plus 0.6µLcitric acid by the end day. The last transaction was then shot twice daily for the remains30 daysinterm.

4- Test two (n=20) driven by physiological saline, citric acid (solvent) and heroin injected in a term of 33 days with the following order: from day 1-3 transaction of 60mg kg^{-1} dosage for independency to heroin[6] to make an injection of 1.15 mL physiological saline, 1.15 mg heroin and 0.003 mL citric acid. Solution was dosage 3 times per day; in the first one was 165µL in the next day, 195µL in the later225µL The last transaction was then shot twice daily for the remains 30 days in term. (heroin dosage of thistestband was 5 mg kg^{-1}).

5- Test three (n=20) transaction done through physiological saline, heroin and acetic acid by shot with dosage of 5 mg mL^{-1}in a term of 33 days. In this band the order of dosing the solution was like to that of the 3 rd.band.

Six members from each band whom showed signs of kidney failure were chosen, weighing and serial urinalysis before nephrectomy carried out after anaesthetizing the animals[6].

Physiological biomarkers fulfilled by excellence balance (METTLER TOLEDO- Columbus) for evaluating body weight, clinical chemistry analyser (RX series - Randox co. UK) for estimation each of creatinine-protein-urea, microscope(Carl Zeiss, branded as ZEISS, Germany) for histological reasons, equipment and tools PerkinElmer, Inc., USA for the rest of parameters.

Histology sections got from fixing fresh kidneys in 10% natural formalin before embedded with paraffin. Then each 4-6-µm-thick section was dyed by both of Masson's Trichrome and periodic acid Schiff. Deparaffinization accomplished by rinsing and oxidizing in a solution of 0.5% periodic acid. Later, sections incubated and counterstained in Schiff reagent and hematoxylin subsequently. For Masson's stain, Bouin's solution was used for deparaffinization and refixing for 50 min. at 58–60°C. Later staining applied with Biebrich scarlet-acid fuchsin and Weigert's hematoxylin before ending dying with aniline blue[7].

Statistical methods: accomplished by applying One-way ANOVA analysis of variance plus complementary Post Hoc Tukey (Honestly significant difference) test[8].

Results

Tabulated **clinical signs** of kidney injury listed below in table-1. Showing obvious decrease in weight accompanied with declines in each of feed intake, water drink, and urine volume values. Concealed datum appear just in cases of feed-water intake without significant differences after applied statistical analysis. Scheme histogram-1 designed below distinguishes the levels of these four parameters according to the tested rat groups showing a direct proportional relationships.

listed **clinical chemistry** parameters recorded in table-2. Obvious elevation in each of creatinine, proteinuria, protein/ creatinine and urea lab values. The only decline appeared in creatinine clearance level. Statistical significant results appear in all of these variables. Statistical histogram-2 below explained that direct proportional relationships correlates each of these variables with the increased dose of the applied heroin.

Table-1. Quantitative physical assessed parameters in heroin tested bands of rats.

Parameter	Standard	Control	T1	T2	T3	p
Weight (g)	250.1 ± 11.1	266.7 ± 18.2	241.0 ± 20.1	235.2 ± 22.5	220.0 ± 23.1	0.15
Feed intake (g)	25.7 ± 1.3	24.7 ± 2.2	21.9 ± 2.6	20.7 ± 3.1	19.9 ± 2.9	0.11
Water intake (ml)	25.1 ± 4.3	24.9 ± 4.4	22.5 ± 5.2	21.5 ± 6.3	20.5 ± 5.5	0.15
Urine volume (ml)	12.1 ± 1.2	11.5 ± 2.9	9.8 ± 1.4	9.1 ± 4.2	7.6 ± 2.4	0.09

* No significant difference explained

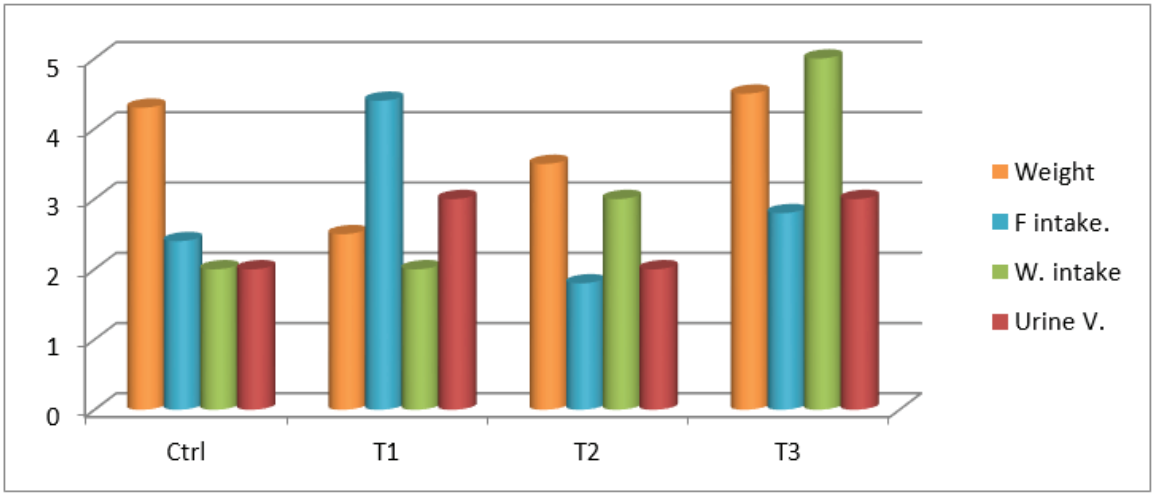

Figure 1: Statistical histogram of physicalvariables related to rats treated by heroin

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kidney injury: Tubular destruction convoyed cell injury of the lethal and sublethal type. Necrosis as coagulative ones leading to apoptosis, and cellular exfoliation in the renal tubules. Other obvious signs including swelling, focal necrosis, acute apoptosis, desquamation, proximal tubules dilation, thinning of PAS+ brush border, granular and coarse pigmented cases, hyaline casts of Tamm-Horsfall protein (urinary glycoprotein normally secreted by eosinophils), leukocytes and interstitial edema were been diagnosed microscopically through all renal tubules slide's sections. Look below shape-1 and -2.

Table-2. Clinical chemistry assessed parameters in heroin tested bands of rats

Parameter	Standard	Control	T1	T2	T3	p
Urine creatinine (mg/dL)	79.9 ± 8.9	82.4 ± 11.2	87.9 ± 10.1	94.4 ± 16.6	102.1 ± 19.2	0.4
Serum creatinine (mg/dL)	0.45 ± 0.015	0.48 ± 0.05	0.50 ± 0.05	0.61 ± 0.03	0.70 ± 0.01	0.01
Creatinine clearance (ml/min)	1.4 ± 0.1	1.42 ± 0.5	1.37 ± 0.4	1.11 ± 0.1	0.88 ± 0.2	0.018
24-hour proteinuria (mg/dL)	7.2 ± 1.6	7.9 ± 5.7	8.1 ± 4.1	9.9 ± 5.1	12.2 ± 4.9	0.60
Protein/creatinine ratio	0.50 ± 0.01	0.66 ± 0.1	0.92 ± 0.4	1.19 ± 0.53	1.70 ± 0.41	0.49
Urea (mg/dL)	29.0 ± 1.9	33.0 ± 3.3	35.1 ± 3.9	37.5 ± 4.4	39.9 ± 5.1	0.059

* No significant difference explained

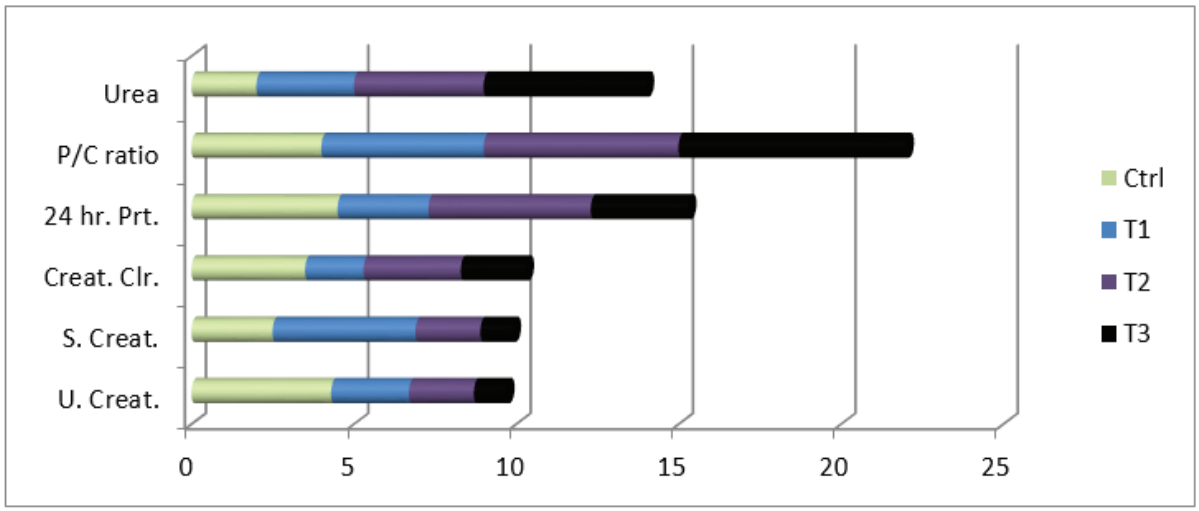

Figure 2: Statistical histogram of chemical variables related to rats treated by heroin.

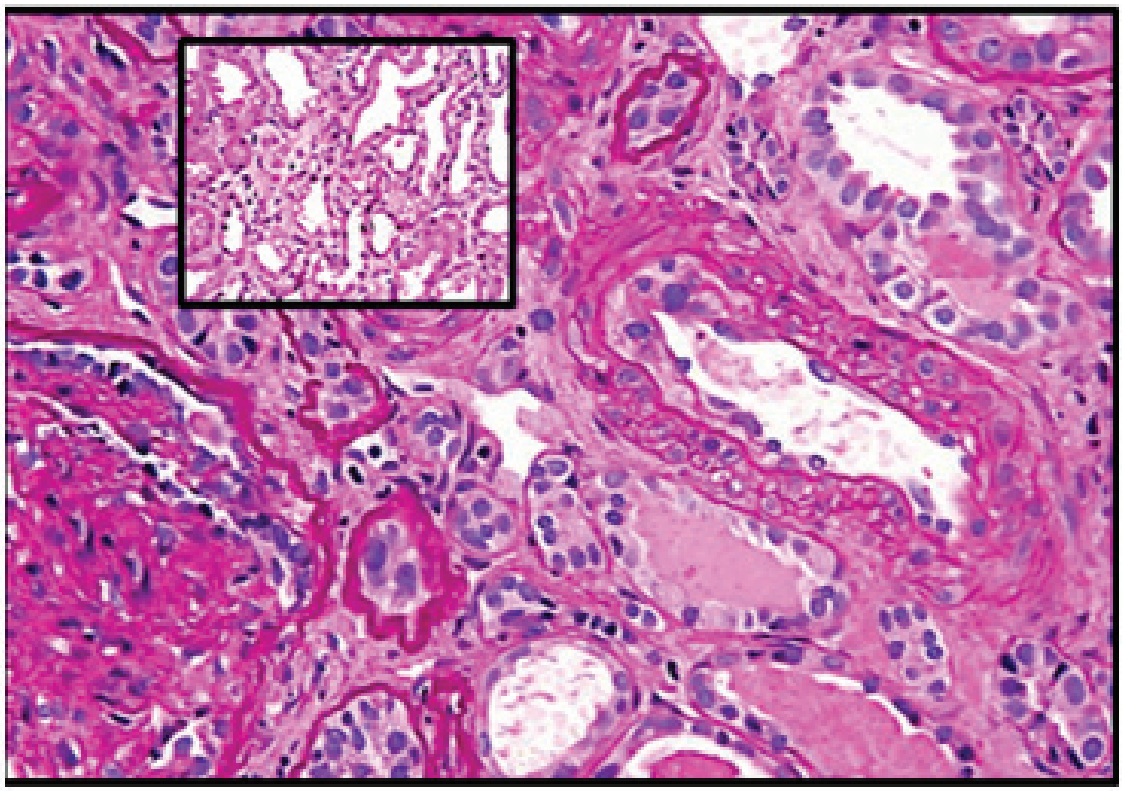

Figure 3: necrosis leading to apoptosis with exfoliation in some tubular parts of the kidney.

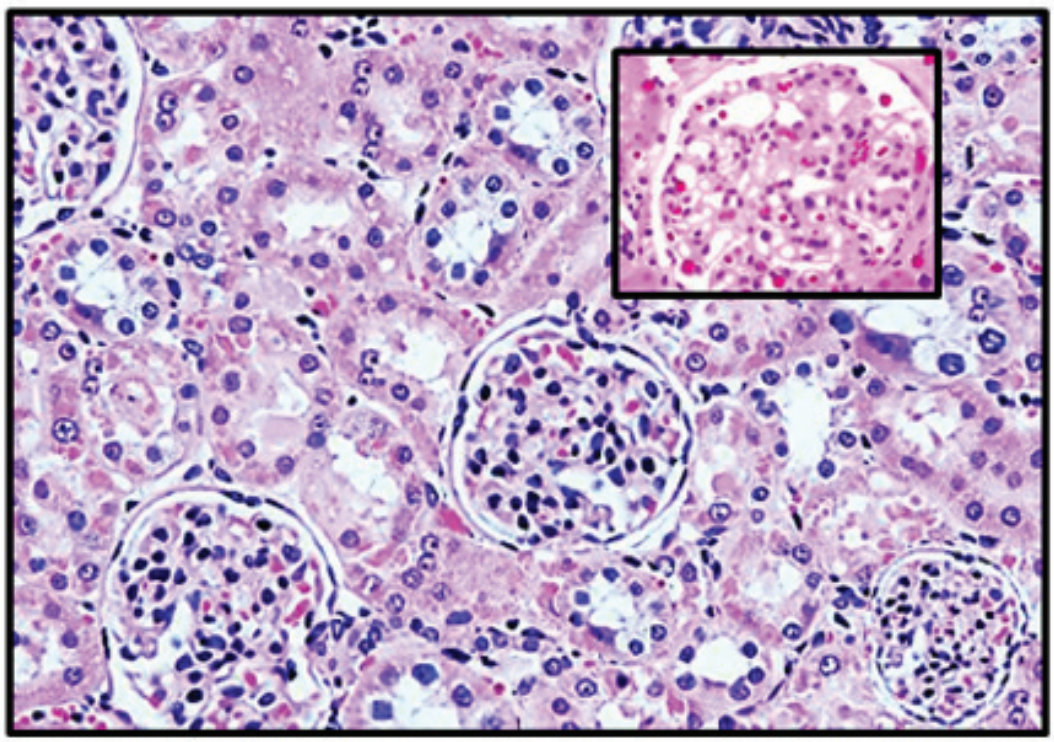

Figure 4: desquamation accompanied with leukocytosis and pigmented destructed corpuscles.

Discussion

Heroin sways rat's kidney injury viaintensificationof protein in tissues involved with inflammation step forwardto kidney failure inducing a dose-dependent raise in albumin excretion and serum creatinine concentration[9].Hence we establisheda significant inflate in creatinine concentration in test-3. So this representationprescribed that increase in concentration of serum creatinine preceded by elevation of proteinuria[10].

High dose of heroin can intensify the hazards of kidney tubulointerstitialdestruction through rhabdomyolysis, which can spot proteinuria *per se*involved directly to this type of damage[11].As another option, proteinuria powereffectively enlightened degradation of kidneyphysiologyviainitiationof hypercholesterolemia, about30days after injection[12]. Supposed toxicity by both of hyperlipidemia and proteinuriahavebeingamechanisms of choice for renal injuryafter suggestive ablation in this rats.

Heroin sometimes linked to liver failure, that may be sway renal physiology through subsequent hypertrophy of glomerulus leading to a suggestive hyperfiltrationas a sign of renal disease[13]. The largevacuolatedrenalepithelium was related strongly with huge proteinuria resulting by case of hyperfiltration, in glomerular sclerosis conditions. Long-term consume of heroinmaytriggerpalliative neuropathy, an irreparablesyndrome that can developed into kidneyinjury, or chronic casefailure[14].

Histological characteristics of our present models distinguished by verdicts of a scattered spreading of destructed and non-destructed renal tubules, overlapped intensively with normal renal unaffected tissues.intermingling of both destructed and non-destructedrenaltubules is features of many advancing kidney illnesses in all laboratory studies and can be assist in understanding the pathogenesis of disease by taking in part that destructive epithelial cells will secrete many growth factors and cytokines[15]. The histological nature of these affected tubules looked atrophic without appearance of markers specialist with distal tubular, but some slides showed supposed remnants of a plasma membrane specializations. This is like to be parts of the otherproximal tubules so[16]. Verifying the exact diagnosis will depend upon using modern histoimmunological techniques for revealing of specific

antigens to proximal tubular cells in the epithelial cells of the atrophic tubules.

Another impressive histological verdict was that manifest affected dilated tubules after injection by 10 weeks. The most prominent signs of these slides including tubular dilation, tubular atrophy, tubulointerstitial fibrosis, with formation of different cast formation that has been considered the noticeable of end-stage renalinjury[17]. A suggestive disruptive physiological modifications in addition to raised chemical metabolism in the hypertrophic (dilated) tubules leading to probable tubular atrophy developed subsequently to tubulointerstitial scarring, this is based on conclusive scientific evidences[18]. The tubulointerstitialpathologicalchanges supposed in the instant model incorporated outwardly unconstrainedbloomingof both large dilated tubules and atrophic tubules. Atrophic tubules case were affiliated with a PCNA-positive cells and smack-like-thickened basement membrane, while the dilated tubules cases seemed to be related to being originating in the spaces of distal tubule. Another noticed observations are that atrophic tubules were abundantbetween5 to 7 weeks after injection of heroin, while dilated tubules numbers being many after 10 week of transaction[19].

Conclusion of this proposal is that heroin doses have gradual and significant impacts on the kidneys physiology through obvious destruction and injury in the renal tissues.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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