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Histological and biochemical effects of diclofenac sodium in adult mice

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ABSTRACT

This study was designed to investigate the adverse effects of a daily single injection of Diclofenac sodium (DS) at two doses on some histological and biochemical parameters in adult mice. The study conducted on 15 of adult mice. Animals were divided into three groups, consisted of 5 animals. Group (I) served as control group and received distilled water only, while group (II) has been set as treated injection with diclofenac sodium 5mg /kg bw for 21 days, group (III) has been treated with diclofenac sodium 10 mg/ Kg bw for 21 days. The following parameters were studied in experimental groups included the histologically effect on kidney, and liver, biochemical tests to evaluated the activity of the enzymes aspartate aminotransferase (AST) ,alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) . The results showed that Ds at dose of 5 mg / kg and 10 mg /Kg induce a significant increase in the level of the activity of the enzymes , (AST ,ALT and ALP) at p value $P<0.05$. DS showed significant alteration in kidney and liver tissues compared to control group, for instance ,histological studies in mice treated with DS (5mg /kg)showed massive aggregation of lymphocytic and other white blood cell (WBC)in their kidneys ,also hepatic tissues treated with DS(5mg/kg)were clearly affected and showed hypertrophy of parenchymal tissues and sinusoids were narrowing with kupffer cells. Similarly ,sever and marked damage were found in mice kidney, and liver tissues after treatment with DS(10mg/kg). Overall, these results revealed the toxic effect of DS at 5 and 10 mg /kg on mice kidney ,and liver

1. Introduction

Over 50 years ago, inflammation is treated by non-steroidal anti-inflammatory drugs (NSAIDs)that are one of the most widely used drugs in medicine and their uses has dramatically increased in recent years. They are a class of drugs that reduce pain and decrease the inflammation [1,2].

Diclofenac sodium is abenzene acatic acid derivative, the chemical name is 2-1 (2,6-dichlorophenyl) amino benzene acetic acid, which has molecular weight 318.4.It has power against COX-2 which is considerably more prominent than that of indomethacin and different NSAIDs[4] .It is abenzene acatic acid derivative, the chemical name is 2-1 (2,6-dichlorophenyl) amino benzene acetic acid, which has molecular weight 318.4.The selectivity of diclofenac for COX-2 resembles that of celecoxib. In addition, it appears to reduce intra cellular concentrations of free amino acid in leucocytes, perhaps by altering its release or uptake, Also diclofenac has anti-bacterial action shown by inhibition of DNA synthesis [3,4]. In the present study was carried out to investigate the histological and biochemical changes in the liver, and kidney, of mice exposed to high doses of diclofenac sodium.

2. Materials and Methods

2.1 Experimental Animals

An adult mice, it has been achieved in the animal house of the college of veterinary medicine/Tikrit University. The animals were maintained under controlled environmental conditions. They were provided a free access to standard pellet diet and tap water. The animals were divided into 3 groups each group consists of 5 animals:G1control group ,G2 treated with diclofenac sodium 5mg / Kg b.wt and G3 treated with diclofenac sodium 10mg / Kg . 3-2.

2.2 Blood collection

Blood samples were collected in dry centrifuge tubes for serum preparation, sera were separated and preserved at -20°C till used for biochemical analysis to detect aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ad alkaline phosphatase (ALP) levels which measured by spectrophotometric kit.

2.3 Histological study :

After sacrifice, livers, kidney and brain were obtained from the mice and immediately fixed in 10% formalin. The tissues excised and covered with physiological normal saline and cleaned from attached fat and connective tissue. Blocks of tissues were immediately fixed in 10% neutral buffered formalin, dehydrated with graded series of ethyl alcohol and embedded in paraffin. Photomicrographs of the stained slides were taken using digital camera attached to light microscope [5,6].

3. Results:

3.1 Biochemical parameters:

Concerning the liver biochemical parameters in [Table 1], AST and ALT, ALP, were highly significantly increased in treated group compared with control group.

Table 1: Effects of diclofenac sodium on the serum ALT, AST, and ALP in adult mice.

Parameters	Control	DS (5mg/kg)	DS (10mg/kg)
ALT(IU/L)	31.32±7.74	75.23±2.85	129±5.14
AST(IU/L)	40.28±1.76	80.65±2.61	169 .33±2.61
ALP(IU/L)	136.18 ± 1.81	148.7 ± 2.94	258.53 ± 1.83

Results represent mean ± standard deviation of group serum results obtained P<0.05.

3.2 Histopathological examination:

Our findings showed some histopathological changes in the kidney, liver and brain after 21 days of study.

3.2.1 G1 /control group ,The Kidney :-

In the control group the structure of Kidney, The cortex of the kidney was containing the glomeruli, which was appeared normal in shape as a tuft of capillaries inside Bowman's capsule surrounded by capsular space.

The proximal convoluted tubules were present with distal convoluted tubules around the renal glomeruli with its lumens (Fig.2, 3).

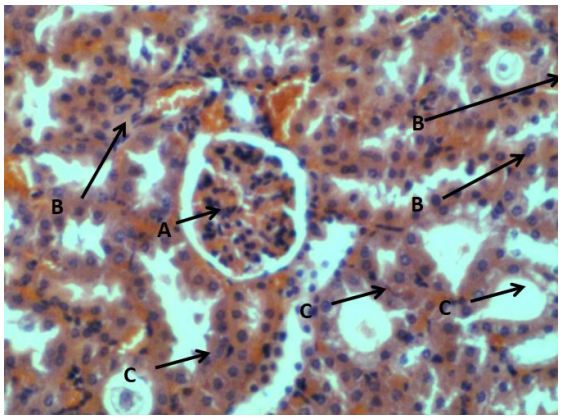


Fig (1): Renal cortex, Demonstrating the shape of glomerulus (A), surrounded by a great number of proximal convoluted tubules (B)and Distal conv. tub .(C). (H & E ×20).

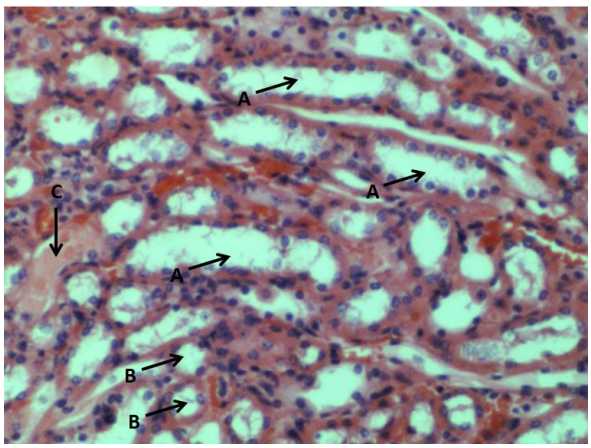


Fig (2): (control) Renal medulla : Renal tubules (A). Henle's loops (B),Blood vessels (C).(H &E ×20).

The renal medulla was containing the renal tubules and collecting ducts with Henels loops and blood vessels were present in between (Fig.3).

Control Liver:-

The parenchyma of the liver was formed by hepatic lobules ,each lobule was formed by Central vein in the center and surrounded by hepatic cellular cords or columns which are present honey-comb like with present of spherical nuclei inside each cell .these cells are surrounded by sinusoid which was containing Kupffer cell(Fig.3).The portal area were containing vein ,branch of hepatic artery ,Bile duct and lymph vessels.(Fig.3).

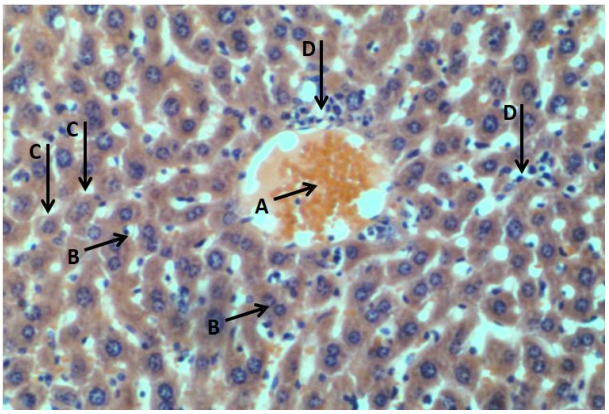
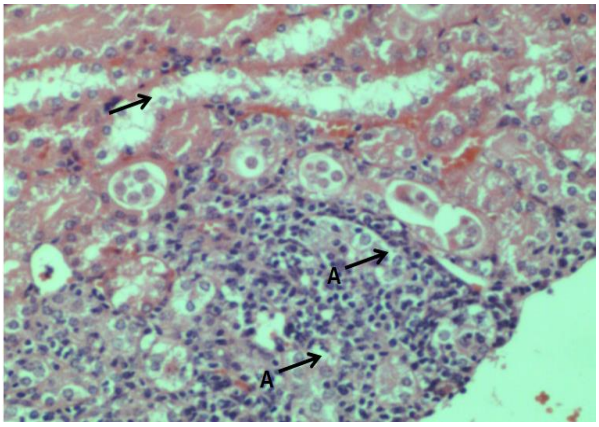


Fig (3): Hepatic lobule ,Central vein (A) hepatic cells (B),Blood sinusoid (C), Kupffer cells (D). (H &E ×20).

The Kidney :-

The cortex of the kidney was containing atrophied glomeruli and segmented ,so the capsular space was wide .The most of the proximal and distal convoluted tubules were containing desquamated epithelial cell from its luminal surface. The renal medulla was characterized by the presence of massive aggregation of lymphocytic and other WBC inflammatory cells in the pelvis of the kidney and around the renal tubules ,which also appeared containing desquamated cells in its lumens (Fig 6,7).



Fig(4): Renal medulla ,showing massive aggregation of lymphocytic and other WBC in the pelvis (A). (H &E ×20).

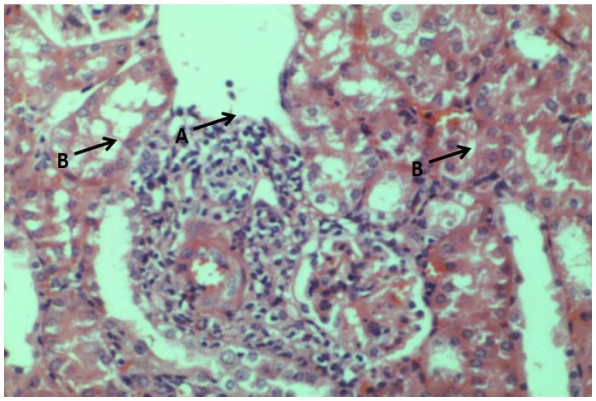


Fig (5): Lymphocytic nodular aggregation (A) in the renal medulla ,Desqumated cells in the lumen of renal tubules (B). (H &E ×20).

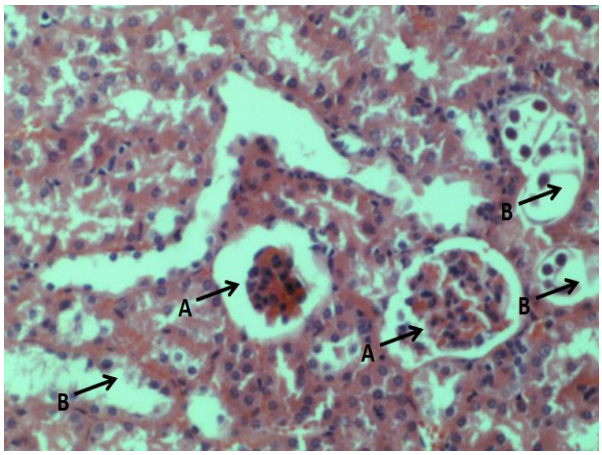


Fig (6): Atrophied glomeruli of renal cortex (A).Desquamated cells in the lumen of proximal and distal tubules . (H &E ×20).

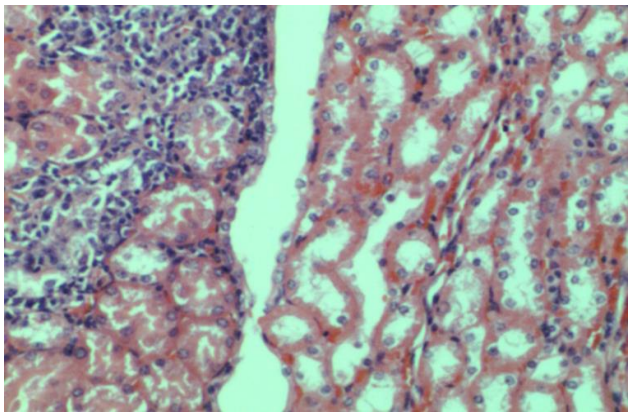
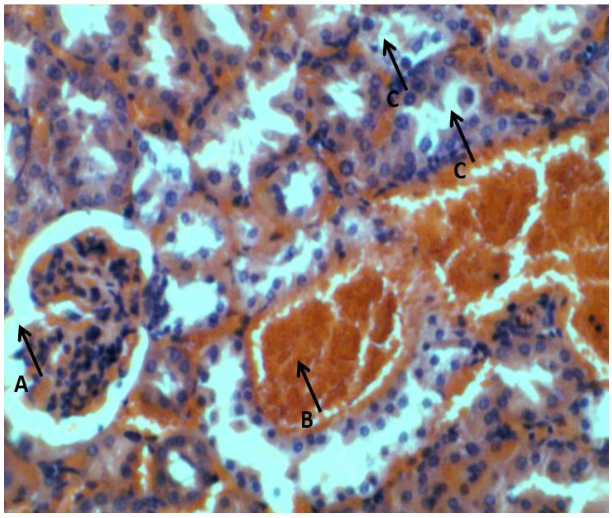


Fig (7): Renal medulla ,showing lymphocytic and other inflammatory cell in between renal tubules . (H &E ×20).

G3/ This group receive indomethacin 10 mgLkg bwt .

The kidney :

The renal cortex was containing atrophied glomeruli ,with partial segmenation .The blood vessles of cortex a round the glomeruli and convoluted tubules were severly congested with blood ,renalmedulla showed thickening of wall of Henles loop. (Fig 9,10).



Fig(8): renal cortex atophied glumerulas with partial segmentation (A). svere congestion of B.V (B). : desqumated cells of proximal and distal convoluted tubules (C) (H&E ×20)

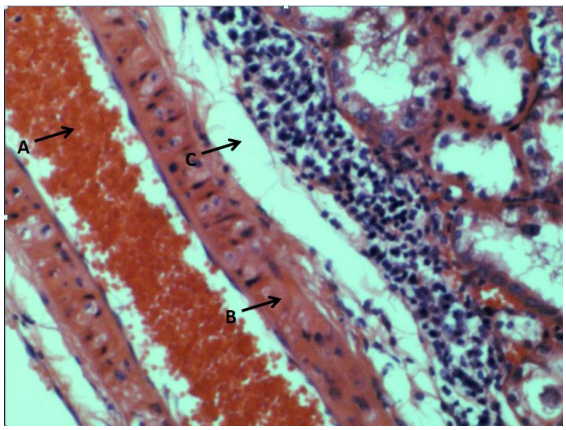
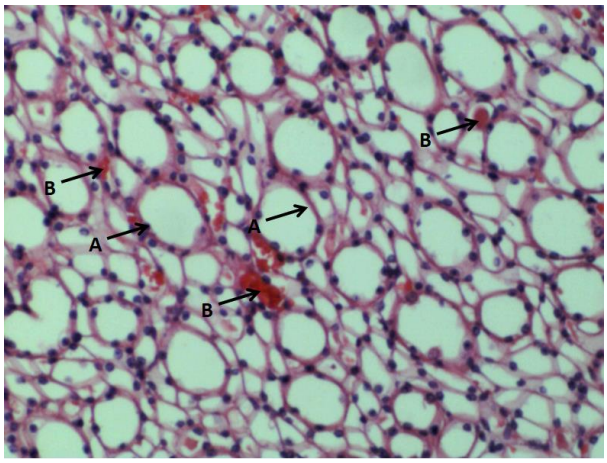


Fig (9): Renal medulla ;congestion of blood vessles with blood (A)Thickening of blood vessles wall (B).Sheath of lymphocytic infilteration a round blood vessles (C) (H &E ×20).

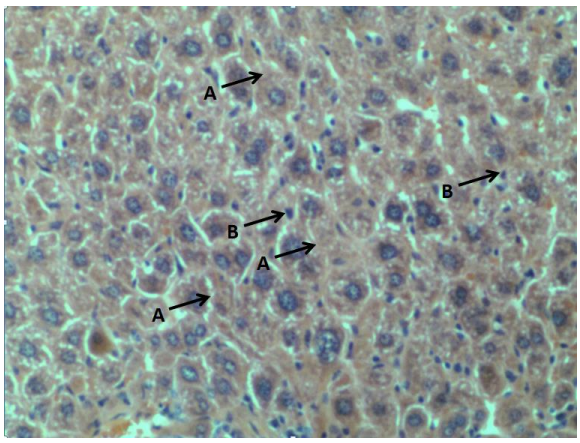


Fig(10): Renal medulla showing thickening the wall of Henels loop (A) . Minut blood capillaric with blood in between it .(B) (H &E ×20).

G2 / Liver /5 mg /Kg BW

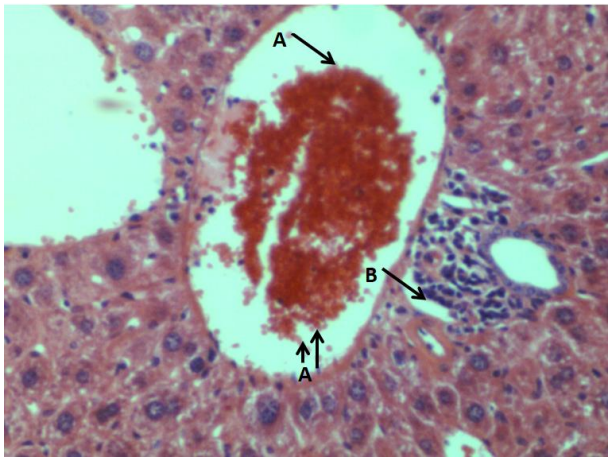
The parenchyma of the liver was contains irregular shape of liver cells ,tend to be round instead of polygoral –shape,and the cells in this group were hypertrophied , its nuclei were karyolitic or karyohexic ,also some of liver cells appeared deroid for nuclei (degenerated and necrotic cells).

The sinusoids were narrow and kupffer cells inside it were demonstrated well (Fig . 11,12).



Fig(11): Liver parenchyma demonstrated hypertrophy of liver cells (A),Kupffer cells (B) in the sinusoid (H &E ×20).

The central vein seen congested with blood partially ,and surrounding by lymphocytic mass infiltration ,(Fig. 11).



Fig(12): Congestion of blood inside the central vein of hepatic lobule (A).Lymphocytic mass infiltration around central (B (H &E ×20).

G3 / Liver /10mg /Kg BW;-

The parenchyma of the liver was degenerated the lymphocytic infilteration around the portal vein ,in the portal area (Fig12). The blood vessels of this area of the sections had homogenous blood (hemolysis) and the presence of WBC inflammatory cells to be forming sheath –like around the bile duct of the portal area. The surrounding liver cells appeared atrophied and some of them were degenerated with Karyorrhexis nuclei . (Fig 13,14).

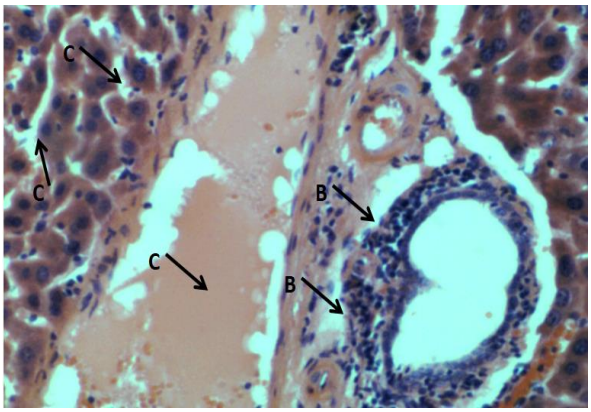
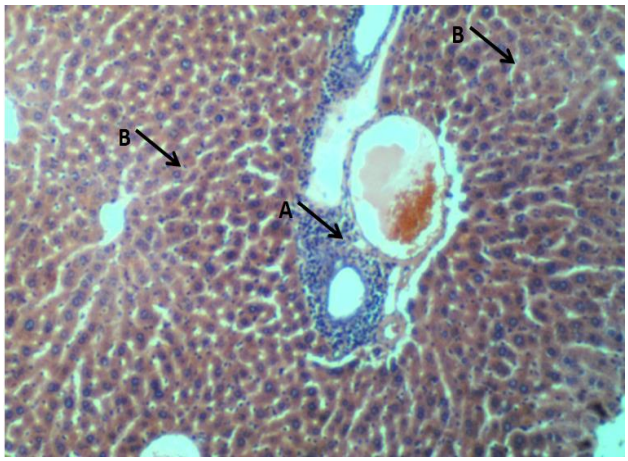


Fig (13): Hemolysis of RBC(A)inside the portal vein in the ,sheath of lymphocytic atrophy of liver cell (c).(H&E ×20).



Fig(14): Liver parenchyma demonstrating lymphocytic infiltration around portal vein in the portal area (A),liver cells (B) (H &E ×10).

4. Discussion:

Diclofenac sodium is the most frequently prescribed therapeutic agents, used for the treatment of rheumatic diseases, because they have analgesic, antipyretic and anti-inflammatory actions. The findings of the present investigation showed that administration of diclofenac sodium at dose of 5 mg/kg b.wt for successive 21 days to mice, caused a significant impairment in liver, kidney and brain functions during the period of experiment. As, serum ALT, AST and ALP activities were significantly elevated level compared to their corresponding values in control group (group 1) and the other treated one (5 and 10 mg/kg b.wt), due hepatocellular damage or necrosis of the liver cells leading to leak out into the blood circulation, drastically increasing their levels in blood.

In current study the administration of diclofenac sodium at dose of 5 mg/kg b.wt for 21 days to mice, caused increase in the activities of AST, and ALT in serum might be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream[7].

The mechanisms of diclofenac induced hepatic idiosyncratic adverse drug reactions remain largely unknown [8,9]. Three metabolites of diclofenac sodium are reported

to be responsible for diclofenac sodium toxicity in the liver, namely 4 hydroxy 3 diclofenac, 5 hydroxy 4 diclofenac and 5 hydroxy 6 diclofenac [10,11]. Both the formation of a toxic metabolite and covalent binding of the drug to hepatic proteins have been invoked to explain its toxicity. Inhibition of prostaglandins is the most important cause of the adverse effects of diclofenac on kidney, liver and brain tissues in sensitive persons or animal species and potentially during long term use[12,13]. The kidneys are involved in the secretion of several, toxins, and regulate the volume and composition of the extracellular fluid to maintain homeostasis by constantly processing the plasma by filtration, reabsorption, and secretion of substances, thereby help in preserving the internal environment of the body[14].

Histopathological examinations showed severity of the lesions increased with increased the dose of drug, the renal medulla was characterized by the presence of massive aggregation of lymphocytic and other WBC inflammatory cells in the pelvis of the kidney and around the renal tubules ,which also appeared containing desquamated cells in its lumens (Fig 7,8,9) in mice which treated with diclofenac sodium with dose 5mg/kg .The cortex of the kidney was containing atrophied glomeruli and segmented ,so the capsular space was wide .The most of the proximal and distal convoluted tubules were containing desquamated epithelial cell from its luminal surface (Fig 8) in mice which treated with diclofenac sodium with dose 10mg/kg . The mechanism of kidney destruction because of the oxidative stress involves the secretion of cytokines, mainly tumor necrosis factor TNF-α, interleukin IL-1, and IFN-c. These findings agree with those obtained by EL-Hamammy *et al*[15] Hickey et al. [16]and Aydin [17] in mice, rats and rabbits respectively. Also Hussain *et al*., [18] observed some histopathological alterations in liver , and kidney of broiler birds and pigeon respectively treated with diclofenac sodium.

Conclusion

Our findings pointed out the risk of renal, and hepatic tissues damage due to long term use of diclofenac sodium . Although this diclofenac sodium has been reported as drug effective in pain management ,their toxic effect must be kept in mind during chronic usage.

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دراسـة التأثيـرات النسيجية والكيموحيوية لدكلوفيناك الصوديـوم في الفئـران البالغـة

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الملخص

صممت هذه الدراسة لمعرفة الآثار السلبية للحقن الفردي اليومي لديكلوفيناك الصوديوم (DS) بجرعتين على بعض المتغيرات النسيجية والكيميائية الحيوية في الفئران البالغة. أجريت الدراسة على 15 من الفئران البالغة. قسمت الحيوانات إلى ثلاث مجموعات ، كل مجموعة 5 حيوانات. استُخدمت المجموعة (1) كمجموعة ضابطة وتناولت الماء المقطر فقط ، بينما تم تعيين المجموعة (II) على أنها حقنة مُعالجة باستخدام ديكلوفيناك صوديوم 5 مجم / كجم من وزن الجسم لمدة 21 يومًا ، وعولجت المجموعة (III) باستخدام ديكلوفيناك الصوديوم 10 مجم / كجم. وزن الجسم لمدة 21 يومًا. تمت دراسة المعلمات التالية في المجموعات التجريبية التي تضمنت التأثير النسيجي على الكلى والكبد والاختبارات الكيموحيوية لتقييم نشاط إنزيمات الأسبارتات أمينوترانسفيراز (AST) والألانين أمينوترانسفيراز (ALT) والفوسفاتيز القلوي .(ALP) أظهرت النتائج أن Ds بجرعة 5 مجم / كجم و 10 مجم / كجم تحفز تنظيمًا كبيرًا في مستوى نشاط الإنزيمات AST) و ALT و .ALP) أظهر DS تغيرًا كبيرًا في أنسجة الكلى والكبد والدماغ مقارنة بمجموعة التحكم. على سبيل المثال ، أظهرت الدراسات النسيجية التي أجريت على الفئران المعالجة بـ 5) DS مجم / كجم) تراكمًا هائلاً للخلايا الليمفاوية وخلايا الدم البيضاء الأخرى (WBC) في الكلى ، كما تأثرت الأنسجة الكبدية المعالجة بـ 5) DS مجم / كجم) بشكل واضح وأظهرت تضخمًا في الدم. كانت الأنسجة المتنيّة والجيوب الأنفية تضيق مع خلايا كوبفر. وبالمثل ، تم العثور على ضرر شديد وملحوظ في أنسجة الكبد والكلى لدى الفئران بعد العلاج بـ 10) DS مجم / كجم). بشكل عام ، كشفت هذه النتائج عن التأثير السام لـ DS عند 5 و 10 مجم / كجم على الفئران.