# Some Anatomical and Histological effects of Diclofenac Sodium to the Stomach, Kidney and Liver in rats.

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# بعض التأثيرات التشريحية والنسيجية لصوديوم دايكلوفان على المعدة, الكلى والكبد في الجرذان.

**المستخلص**

صوديوم ديكلوفان هو واحد من الادويه غير الاستيروديه المضاده للالتهاب وهو شائع الاستعمال لغرض تخفيف الالم وعلاج عدة اضطرابات مثل امراض الكلى والالتهابات والحمى. هذه الدراسة صممت لغرض معرفة تأثير الجرعه الزائدة من صوديوم دايكلوفان 75ملم/كغم من وزن الجسم لمدة خمسة ايام من الناحية التشريحية والنسيجية لكل من المعده والكبد والكليه. واجريت الدراسه على 24 جرذ مختبري (أناث) تتراوح اعمارهم مابين( 8-10) أسبوع . التغيرات التشريحية تضمنت زياده بالوزن وتغيرات في سطح هذه الاعضاء والطبقة المخاطية للمعده ولكن في الكليه ادى الى احتشاء في بعض مناطق القشره واللب وكذلك سبب احتقان فيه, اما في الكبد ادى الى حدوث التهاب بالخلايا الكبدية وزيادة النسيج الليفي.

## Abstract

Diclofenac sodium (DS) is one of Non-steroidal anti-inflammatory drugs (NSAIDs).It is commonly used to relief pain and treats several disorders like renal diseases, inflammation and fever. This study was designed to show the effects of over dose of diclofenac sodium75ml/kg B.W in 5 days anatomically and histologically to the stomach, kidney and liver. 24 experimental rats were used,femal ages range between 8-10 weeks. The anatomical change include increase weight and grossly change in surface of these organs and mucosa of stomach but histological change in these tissue include damages which can form mucosal erosions in stomach. However, in kidney it causes infraction in the same area of cortex. Moreover, medulla causes congestion, and in liver occur hepatocyte inflammation and increase fibrous tissue.

**Key word:**Diclofenac sodium, erosion, infraction, hepatocyte

## Introduction

Diclofenac extend to a chemical subgroup of Non-steroidal anti-inflammatory drugs (NSAIDs) that is an arylalkanoic group of phenylacetic acid (1) The true mechanism is not known but it is further related to the decrease in the fatty acid entering the cell or released from the cell (2). Diclofenac sodium is the most extremely and important used medications for the treatment of a varsity of common acute inflammatory and chronic conditions (3) and

subscribe for use in painful and inflammatory non-rheumatic and rheumatic conditions (4-5). Diclofenac sodium is an inhibitor of cycloxigenase enzyme, it is reduced in leukocyte intracellular free arachidonate level (2). Most prior studies examining gastric mucosal injury have investigated the mechanisms of NSAID prompt gastric mucosal lesions evolution and progression ( 6-7).It has been reported that gastric toxicity is highly effected by the amount of drug dissolved under the pH conditions rather than the force of the drug as an inhibitor of prostaglandin synthesis (8).The capability of diclofenac to cause bleeding and ulceration in the upper gastrointestinal tract was first notarized by the endoscopic (9). Among different disturbances of gastrointestinal system, peptic ulcer is the one which is more prevailing and have greater clinical effect. Ulcer is characterized by disruption of mucosal integrity leading to local defect or hole due to active inflammation (10). Pathophysiology of ulcer is due to an imbalance between attacker factors (acid, pepsin, H. pylori and NSAID) and local mucosal defensive factors mucus bicarbonate, blood flow and prostaglandins (11). (NSAIDs) cause injury to the gastro-duodenal mucosa via several mechanisms inclusive the topical irritant effect of these drugs on the epithelium, weakness of the barrier property of the mucosa, extinction of gastric prostaglandin synthesis, reduction of gastric mucosal blood flow and interference with the reform of superficial injury (12). The presence of acid in the lumen of the stomach also contributes to the path-ogenesis of (NSAIDs) promote ulcers and bleeding by impairing the renewal process interfering with hemostasis and inactivating several growth factors that are critical in the mucosal defense and reform (13).The nephrotoxic and hepatotoxic effects of increasing doses of diclofenac on kidney and liver tissue in rats (14).Diclofenac sodium is intermediate with renal physiology by suppress prostaglandins. Prior studies suggest that various nephrotoxins degenera-tion proximal renal tubules by altering alkaline phosphatase activity it has a function related marker in renal proximal tubular epithelia (15). The effect of diclofenac on the liver is rare but possible its deadly hepatotoxicity that may be correlating with the formation of reactive metabolites (16, 17).The aim of study to focus the over dose of diclofenac sodium it is highly effected to the stomach, kidney and liver and may cause sudden death if the patient has ulcer in stomach.

## Material and Method

In this study we used 24 experimental female rates aged 8-10 weeks divided into two main groups, each group comprised 12 rats, first main group is control group The mean of body weight of control group was 197.08 g and the second main group is treated group the mean of body weight of treated group was 209g .The control group and treated group is subdivided

into two groups, each one consists of 6 rats the first one for anatomical study and the second for histological study. The control groups were injected 1ml of distal water intramuscular. The second main groups (treated groups) are injected diclofenac sodium 75ml/kg B.W over dose intramuscular till 5 days than killed the rates by xylene and make incision in the abdominal wall to anatomical and histological study. The first one subdivided of control group and treated group used for anatomical study of the stomach, kidney and liver to description the gross pathological changes in treated group including shape, color, size and then weighting of these organs and then compared with the normal organs of control group but, the second subdivide of control group and treated group which is used for histological preparation of these tissue of stomach, kidney and liver then transferred of these tissues after washings by water to solution of 10% neutral buffered formalin fixative in marked containers for 48 hours (18). After the fixation completed the specimens were washed using tap water for 2 hours, then the following routine histological steps used Hematoxilin and eosin stain were performed to show the normal and abnormal tissues and then compared between it.

## Results

## Clinical singes

After injection of diclofenac sodium in third days on experimental rates, in cage they indolence, pale eyes, loose of appetite, unhealthy hair, present blood in faces and more clear appear this sings in fifth days and although present of loose of appetite but there was an increase in mean of body weight of treated rats when weighted. The mean of body weight of control group of rats was 197.08 g but the mean of body weight of treated group of rates was 209g.There is a significant different in the mean of the body weight of the rats.

## Gross changing

In the present study, show the results that after injection of high dose of diclofenac sodium 5 days and killed than open abdomen wall there's anatomical change in stomach, kidney and liver compared to control rat .The change in stomach was clearly obvious enlargement in size due to present of abscess and bleeding .Also the surface color of stomach altered from the white color and trancelent ( Fig.1) in control rats to greenish color and reddish in some area (Fig.2), also the line demarcation between glandular and non-glandular are disappeared (Fig.2) in treated rats because present abscess and hemorrhage.Morever, the mucosal surface of stomach in control rat showed yellow to pink color and thin (Fig.3), but in treated rates showed

the mucosal surface light brown, white spot present and increase thickness of mucosal (Fig.4). However, the grossly change of kidney showed alteration of color surface from red to dark red in treated rates due to present infarction (Fig.5).In liver, the grossly change surface color change from brown to drake brown and present few white spot in the surface (Fig.6).

## Weights of stomach, liver and kidney:

The current study noticed that the mean of body weight of the control rats was 197.08 gm but after injection over dose of diclofenac sodium 75 ml/kg 5 days the mean of body weight increased to 209gm. This due to present of hemorrhage, abscess and edema in some organ after injection of diclofenac sodium, like stomach. In this study the mean weight increased from 1.37 gm in control groups to 2.36 gm in treated groups , also the mean weight of liver increased from 5.56 gm in control groups to 7.32 gm in treated groups and the mean weight of kidney increased from 0.50 gm in control groups to 0.63 gm in treated groups, and showed there was a significant differences at $P<0.05$ in all weight measurement in this study (Table. 1) this due to present abscess and hemorrhage in this organs.

**Table 1: Show the means and stander error for the body weight and weight of stomach, kidney and liver to the control rats and treated rats**

| Animals | Body weight mean±SE | Weight of stomach mean±SE | Weight of kidney mean±SE | Weight of liver mean±SE |
|---|---|---|---|---|
| Control rat | b 197.08±0.93 | b 1.37 ± 0.01 | b 0.50 ± 0.01 | b 5.56± 0.01 |
| Treated rat | a 209.0± 1.34 | a 2.36± 0.09 | a 0. 63 ± 0.21 | a 7.32± 0.23 |

## Histological results

The result of this study show that all layer of stomach tissue in control rats all layer of tissue are intacted (Fig. 7), but in treated rats, they are distracted and extend to the glandular layer (Fig.8). There are damage epithelial and focal ulceration in the mucosa and vascular congestion and pointed hemorrhage distributed in large area and high inflammatory cell

infiltration (Fig.9). But the tissue of liver, in control rats, appeared regular and healthy (Fig. 10), but in treated animals showed congestion of hepato-cytes, degeneration of parenchymal cell, enlargement of sinusoid, fibrosis portal to portal tissue proliferation, bile duct proliferation and present hepatocyte with eosinophil (Fig. 11 ,12). In control rats the kidney appeared intact and regular (Fig.13) but in treated animals showed pale pink infarcted kidney in which both glomeruli and tubular (Fig.14) have undergone coagulative necrosis and irregular vein and proliferation wall of artery (Fig.15).

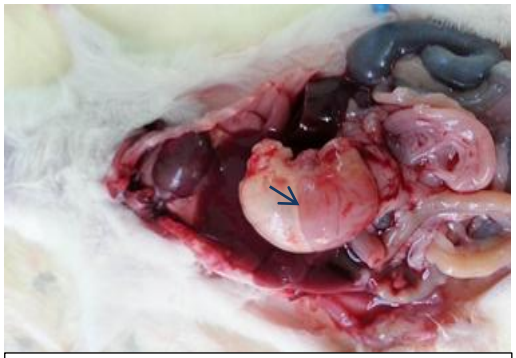

Fig.1: Show grossly normal stomach in control rat, the line demarcation between glandular and non- glandular are clear.

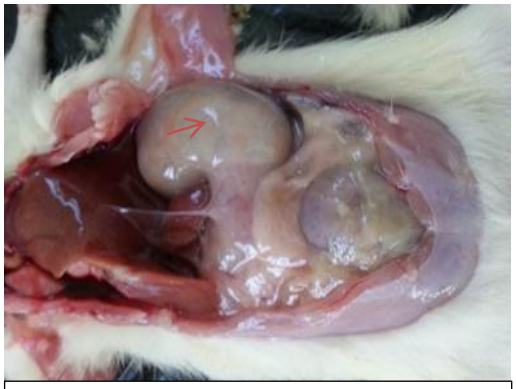

Fig.2: Show the gross anatomy of stomach in treated rat , the line demarcation between glandular and non –glandular disappear and change color surface.

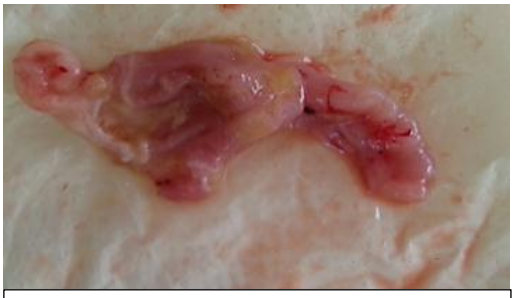

Fig.3: Show the gross anatomy of mucose membrane of control rat.

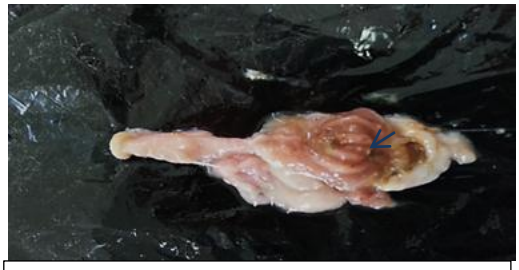

Fig.4: Show the gross anatomy of mucose membrane of stomach in treated rat, there is white pin spotted distributed and change color of mucose membrane to light brown.


Fig.5: A: Show kidney in control rat its red color. B: show kidney in treated rat its dark red color.

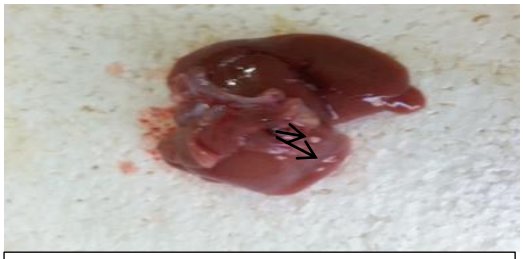

Fig.6: Show the gross anatomy liver in tread rat present few white spot.

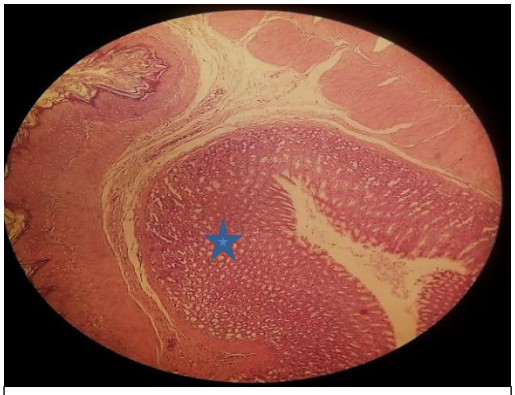

Fig. 7: Show tissue of stomach in control rat intact epithelial tissue. H&E X10

Fig.10: Show tissue of liver in control rat the epithelial is intact. H&E X10

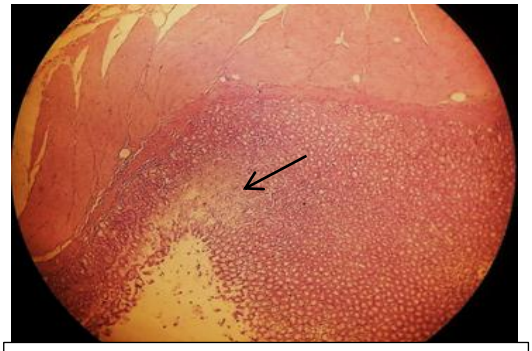

Fig. 8: Show tissue of stomach in treated rat there is focal ulceration in epithelium. H&E X10

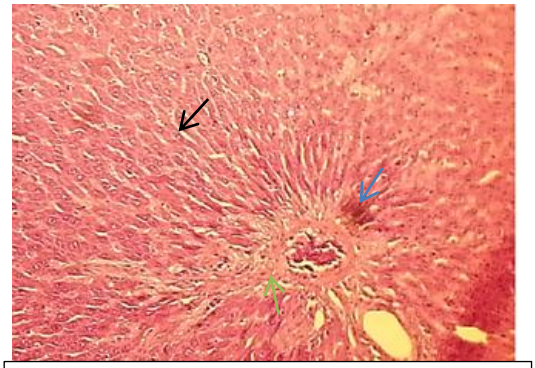

Fig.11: Show tissue of liver in treated rat congestion of hepatocytes (blue arrow), enlargement of sinusoid (black arrow), increase fibrous tissue proliferation around portal vein(green arrow). H&E X10

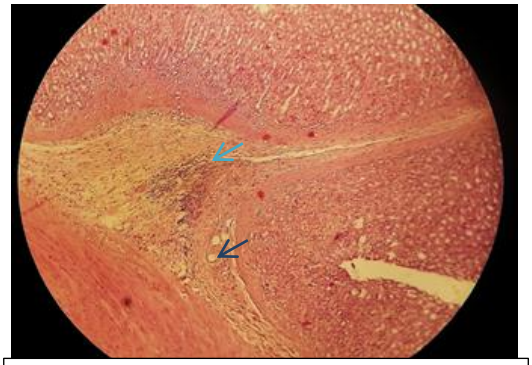

Fig.9: Show stomach tissue in treated rat there is enlargement of blood vessel (black arrow) and high infiltration of inflammatory cells (green arrow). H&E X10

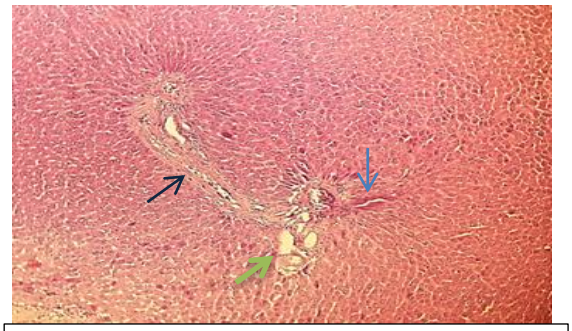

Fig.12: Show tissue of Liver in treated rat degeneration of large area of parenchymal cell (blue arrow), congestion of hepatocytes (blue arrow), fibrous portal to portal proliferation and bile duct proliferation (black arrow) . H&E X10

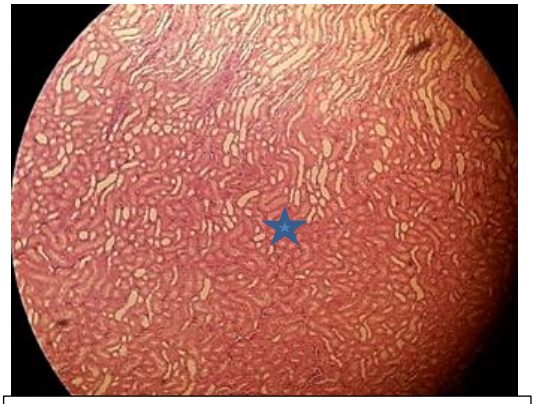

Fig.13: Show tissue of kidney in control rat the epithelial tissue is healthy and intact. H&E X10

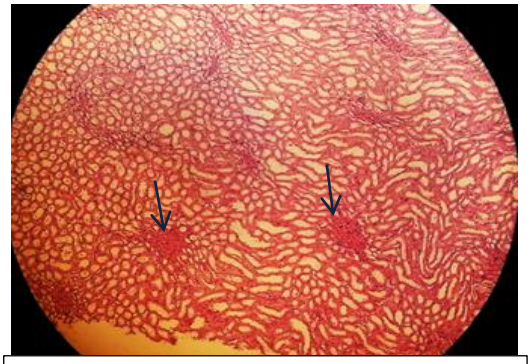

Fig.14: Show tissue of kidney in treated rat show infarcted kidney in which both glomeruli and tubular. H&E X10

## Discussion

A rat stomach which administered of diclofenac, showed grossly pin point hemorrhagic area as well as a wide spread hemorrhag as indicated by the red spots which are blood clots (19), but in this study the stomach clearly showed obvious enlargement in size because present of abscess and bleeding due to excess production of hydrochloric acid also, the surface color of stomach is greenish color and reddish in some areas and the line demarcation between glandular and non-glandular are disappeared, consequent enalargement of stomah and diappear of demarcation as well as white spot present

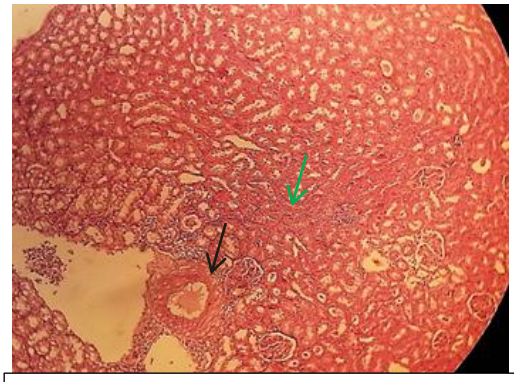

Fig. 15: Show tissue of kidney in treated rat show necrosis (green arrow) and irregular vein and proliferation wall of artery (black arrow).H&E X10

in mucosa surface due to present abscess and increase thickness of mucosal. In liver, there is grossly change in surface it become dark brown and present of white spot in the surface, but the grossly change of kidney showed alteration of color surface from red to dark red in treated rates due to present infarction. In most studies effect of diclofenac is to the stomach, liver and kidney there are very rare anatomical studies to these organs, but in current study, there is an exposure anatomical effect of diclofenac sodium to these organs. But histologyically there are various factors energizing of gastric acid production, cytokines, inflammatory cells infiltration, mucosal blood flow, and free radicals are known to contribute to the development of NSAID induced gastric mucosal damage (20).The diclofenac administration increases gastric damage in a dose dependent manner. (21).NSAID induced gastric damage was different depending on the kind of NSAIDs. diclofenac caused linear-shaped ulcers with dented spots, also the age of rats are effected by the severity of damage cause by NSAID ( 20), but in current study damage occured in epithelium tissue and mucosa and also irregular spot ulcer

occured in mucosa and glandular layer and agree with research which showed necrotic gastric mucosa with severe dilated congested blood vessels in the lamina properia with severe edema infiltrated byinflammatory cell **(**19). The normal dose or high dose of diclofenac sodium both cause damage area in stomach but different in damage area is not in gross ulcer index and induced mucosal damage and neutrophil infiltration in the mucosa ( 20).From the obtained results, it is clear that, using diclofenac in over dose of (75 mg/kg) produces the largest gastric damage, ulcer and hemorrhage.A high dose of diclofenac sodium causes irreversible cell death, also fibrous tissue proliferation, acute cell damage and hepatitis (14, 21 , 22).The association of NSAIDs with liver disease is poorly certified (23), but this study and another study (14) showed that there is a strong relation shape between diclofenac sodium and liver disease because its metabolized in liver tissue and has toxic effect (24). The toxic effects of diclofenac could be acute or reversible (5). The kidney is another organ affected by the toxic effects of NSAIDs (25- 26).The nephrotoxic and hepatotoxic effects of diclofenac sodium in both humans and experimental animals have been reported (27- 28).The toxic effects of diclofenac can be acute or reversible (28). The diclofenac cytotoxicity can be seen as in the condition of chronic pyelonephritis and acute hepatitis (14). The NSAIDs adversely change the kidney functions (2), the Prostaglandins do not play a physiologic role in keep renal blood flux in normal subjects but it plays a role in keep glomerular filtration rate and the vasodilatory impact ( 29) .Some authors mentioned that the high dose of diclofenac caused tubular epithelial cell damage, and necrosis that may be irreversible or reversible also increased fibrous tissue in the interstitial tissue of the kidney (14). Tablet diclofenac 50 mg three times a day (150 mg/day) was adequate to precipitate acute renal failure as this dose appears to reduce the renal blood influx and glomerular filtration rate (14) . Previous researchers indicated that the diclofenac induced kidney damage cannot be estimate by kidney stereological and serum oxidative stress parameter, however, more severe kidney damage may cause appearance change in these parameters (30), but histologically, it can be easy to diagnose and in early stage therefore the histological study is important .Finally, there is dangerous effect of diclofenac sodium to the stomach liver and kidney if it used over dose and may cause cancer because its cause perforation of tissue, also cause hemorrhage and ulcer and must be careful to using.

## Conclusion

The over dose of diclofenac sodium has a strong effect to the stomach, liver and kidney and it has some anatomical and histological effect to these organs and may cause death in some cases because it causes abscess and hemorrhage in these organs and can cause cancer due to formed proliferations of tissue in these organs and must be carefull to use diclofenac and if used, it must be taken few dose and lesser than the normal dose.

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