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Toxic And Histopathological Changes of harmful Effect of Diclofenac Sodium on Some Loose Organs in Albino Rats for Twelve Weeks

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ABSTRACT

The present investigation demonstrated the side effect of (I.M.) injection (75 mg/3 ml/ rat/ week) of diclofenac sodium for 12 week on the histological structure of some loose organs of adult rats. During the study no clinical sings were seen and none of the treated animals died during the experiment. This indicates tolerance of the rat to toxicity of the diclofenac sodium. Only histopathological changes were seen after termination and those were in the liver, kidney, heart, pancreas and stomach. These were as follows, vacuolation and degeneration of liver cells, dilated cortical tubules in kidney, degeneration and vacuolation of islet of pancreas, vacuolation of myocardial muscle cells in the heart and erosion and ulceration of the glandular stomach.

Keywords- *Albino Rat, Histopathological, Toxic, vacuolation & Diclofenac Sodium.*

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) such as voltaren (Diclofenac Sodium), ibuprofen, naproxen, and indomethacin.

Diclofenac Sodium is a potent nonsteroidal anti-inflammatory drug (NSAID) that is used for the treatment of rheumatoid arthritis, osteoarthritis, analgesic and related diseases as well as the prevention of cardiovascular thrombotic diseases.

Gastric ulcer associated with the use of Diclofenac Sodium is a major problem. Many factors such as gastric acid and pepsin secretion, gastric microcirculation, prostaglandin E2 (PGE2) content (Laine et al., 2008) and proinflammatory cytokines interleukin (IL)-1 and tumor necrosis factor (TNF)- (Santucci et al., 1995 ; Appleyard et al., 1996) play important roles in the genesis of gastric mucosal damage, and its subsequent development (Wang et al., 2007 ; Wallace, 2008). It has been reported that

increases in NO synthase (NOS) activity is involved in the gastrointestinal mucosal defense and also in the pathogenesis of mucosal damage (Wallace et al., 2008). Diclofenac Sodium is extensively used as analgesics and anti-inflammatory agents and produce therapeutic effect through the inhibition of prostaglandin synthesis (Klaassen, 2001). Diclofenac Sodium and other NSAIDs block the formation of colon cancer in experimental animals, and there is epidemiological evidence that chronic NSAID usage decreases the incidence of colorectal cancer in humans (Gupta and DuBois, 1999). The toxicity of nonsteroidal anti-inflammatory drugs (NSAIDs) can be directly related to their biliary excretion. For example, Diclofenac Sodium or indomethacin can cause intestinal lesions. The sensitivity of various species to this toxic response is directly related to the amount of these compounds excreted into bile. The formation of intestinal lesions can be abolished by bile duct ligation (Duncan et al., 1994).

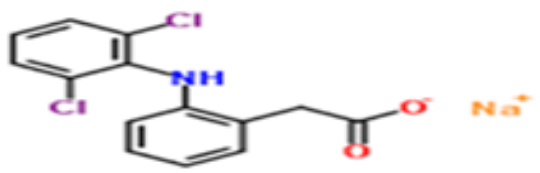


Fig (1): Chemical Structure of Diclofenac Sodium.

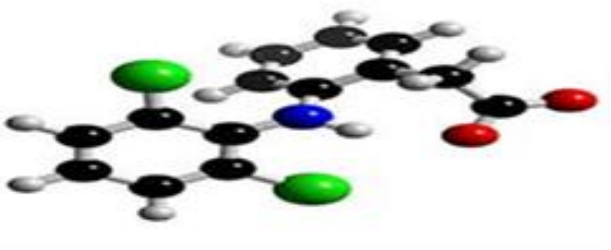


Fig (2): 3d-Structure of Diclofenac Sodium.

2. Aims of the Study

I. To study the toxicological pathology of Diclofenac Sodium by using albino white rats as experimental model.

II. To have an idea about the toxicity of Diclofenac Sodium in different toxic level, to help in avoiding any side effects in human.

III. To explain the high dangerous resulting from consumed too much of Diclofenac Sodium for long time.

IV. To open the way for further research in toxological pathology of Diclofenac Sodium and any related Non-steroidal anti-inflammatory drugs (NSAIDs) for the benefits of human and other veterinary use.

3. Materials & methods

A. ***Animals and design:***

White albino rats of both sexes weighing between 190-230 gm were used for the experiment. They were separated into 2 groups (control and treat), each group consisting of 10 animals, 5 males and 5 females. Then be subjected from December to February 2015. Animals were housed in wire mesh cages under ambient light conditions, with given Diclofenac Sodium I.M. injection in thigh muscle into treat group every week. They were acclimatized to captivity for at least two weeks prior to testing. Diclofenac Sodium (Novartis Pharma Schwiez AG, Switzerland) at 75 mg/3 ml/rat/ week; for twelve weeks. The animals were observed in their cages for clinical symptoms daily. After three months (the end of the experimental period) animals were killed;

and autopsy had been done to histopathological technique procedure and stained using hematoxylene and eosin stains.

B. ***Preparation Organs for Histopathological slides:***

Organs such as the liver, kidney, adrenal, pancreas, cardiac muscle and stomach were isolated into 10% saline formalin for 24 h to fix. The specimens were then embedded in paraffin, sectioned and stained with hematoxylin and eosin, as described by Cook (1973) before being evaluated by light microscopy (Olympus).

C. ***Microscopic examination:***

After sacrificing the experimental animals tissue samples from various visceral organs were taken fix in 10% neutral buffer formalin, then paraffin blocks were made and cut on rotary microtome to make slides of five microns which were then stained with H&E. those sections were examined by light microscope (Olympus) to detect and describe any histopathologic changes induced by the treatment with the Diclofenac Sodium.

4. Results

Results of my experiment summarized by different histopathological lesions; these lesions varied form organ to another but were apparent and considerable in liver and other organs like kidney, adrenal gland , pancreas and heart showed different lesions. The most significant treatment-related histopathological changes were in the liver and those changes were varied from vacuolation of hepatocytes with interstitial edema (figs 2). On occasion those changes were associated with advanced degrees of septal fibrosis (figs 3) also there were centrilobular vacuolation of hepatocytes (figs 4). Furthermore, other toxologic pathologic changes in other visceral organs and tissues were varied from varying degrees of dilatation of renal cortical tubules (figs 6,7) and other changes associated with vacuolation of zona fasculata and reduce size of the adrenal gland (figs 8). In addition further histopathological changes in pancreas as degeneration and vacuolation of islet of langerhans (figs 9) while in heart it was vacuolation of myocardial muscle cells (figs 10), other important histopathological changes were vacuolation, erosion and ulceration in the glandular region of stomach (figs 11,12). The toxic - pathological study for (I.M.) injection of diclofenac sodium in albino rats showed that, the rats is highly tolerant to this material as the long term administration during the experiment. No clinical findings were seen and no death in the experimental animals, only microscopic lesions were detected as above in liver, kidney, pancreas, heart and stomach.

Note: all sections were taken transversely or obliquely from visceral organs of both male and female.

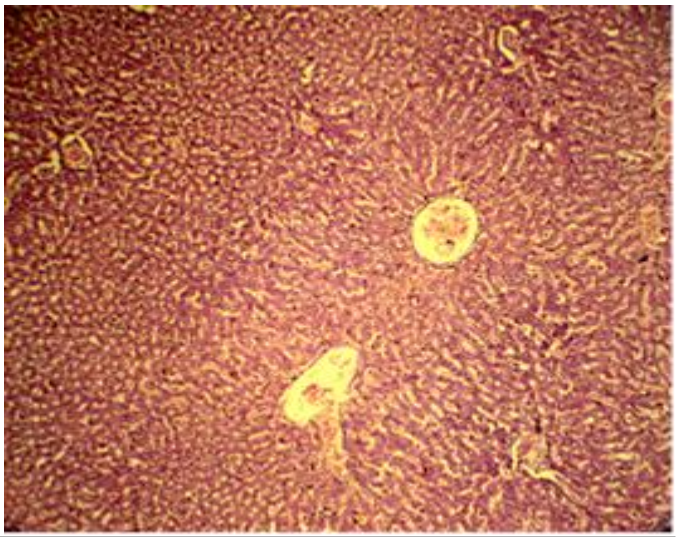


Fig (1): Liver; control, within normal structure. (H & E, 4X)

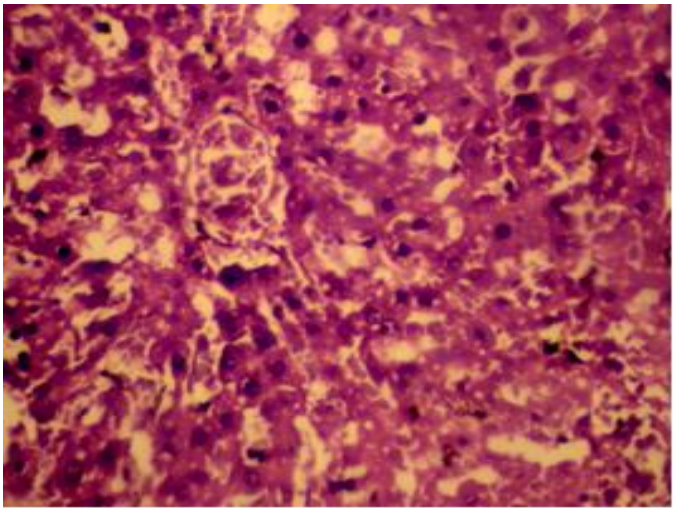


Fig (2): Liver; vacuolation and degeneration of hepatocyte. (H & E, 40X)

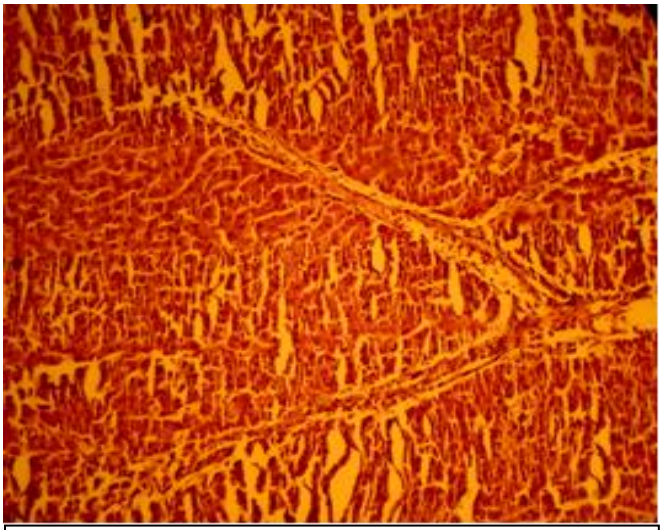


Fig (3): Liver; septal fibrosis with interstitial edema. (H & E, 10X)

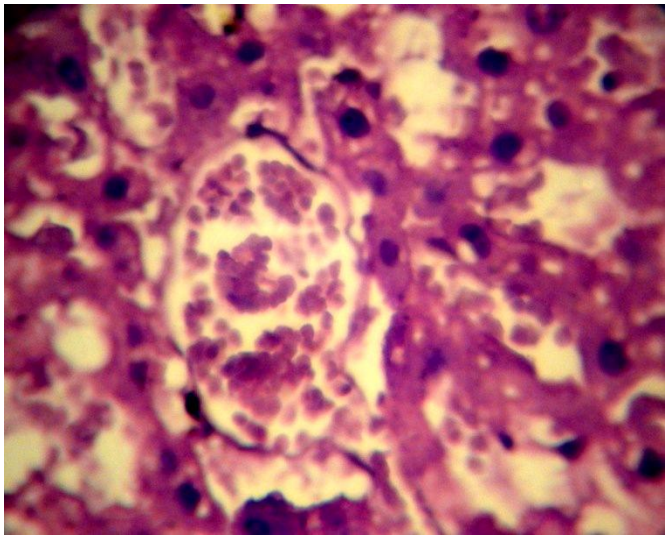


Fig (4): Liver; centrolobular vacuolation of hepatocytes. (H & E, 10X)

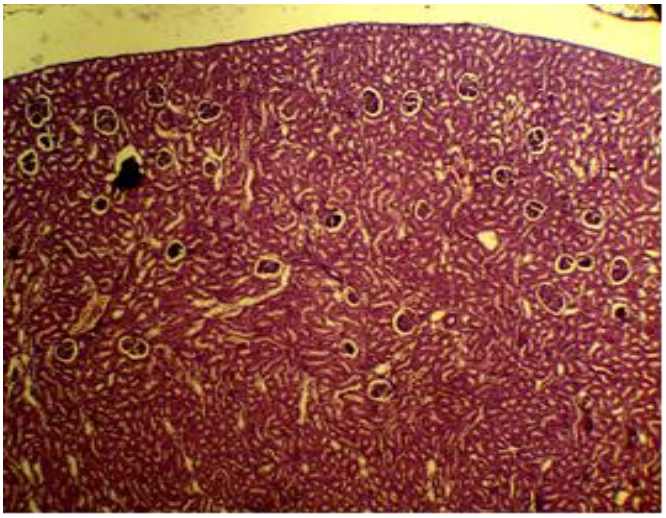


Fig (5): Kidney; control, within normal limits (H & E, 4X)

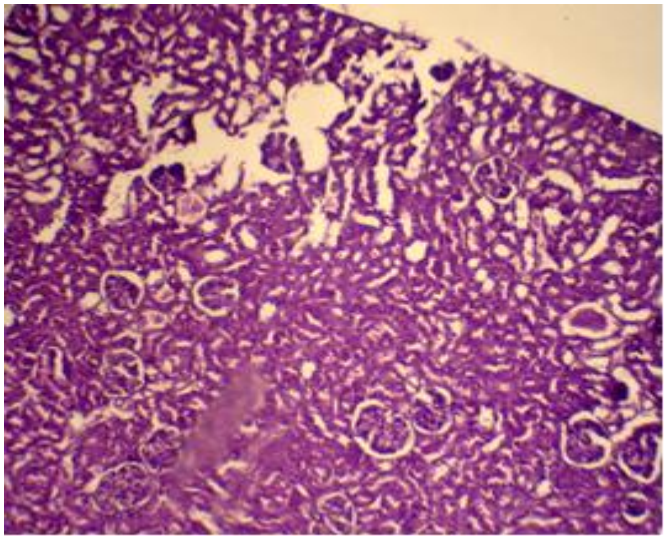


Fig (6): Kidney; marked dilation of cortical tubules. (H & E, 10X)

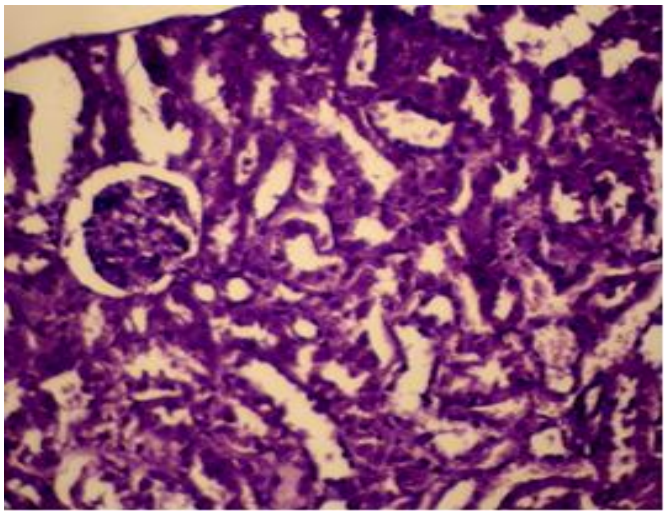


Fig (7): Kidney; vacuolation of glomeruli and area of dilation of cortical tubules. (H & E,40X)

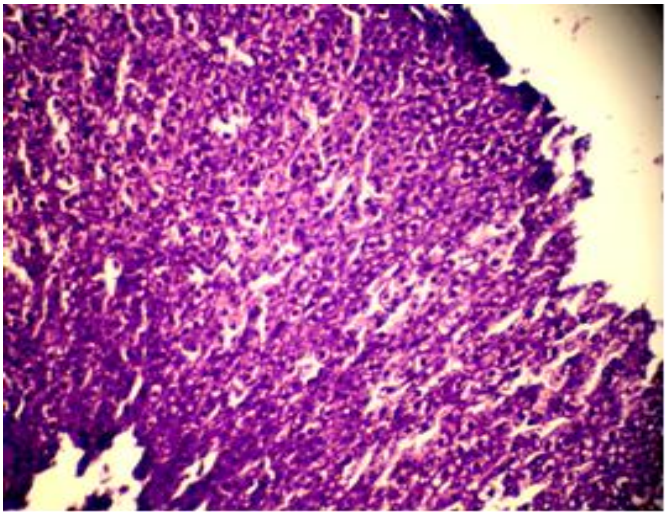


Fig (8): Adrenal gland; vacuolation and degeneration of zona vasculata and reduce in size. (H & E, 10X)

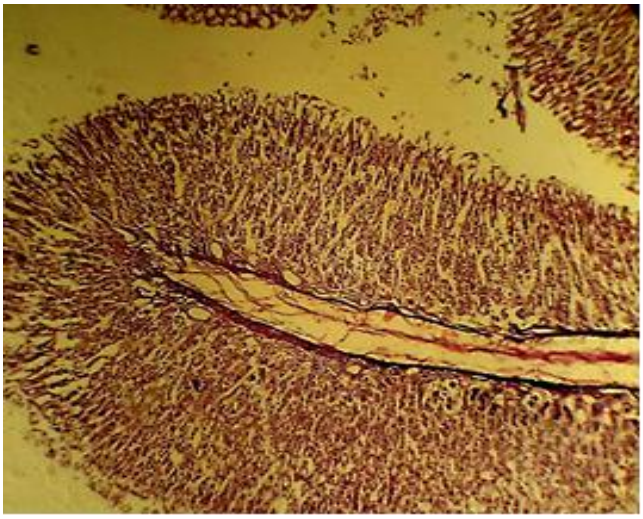


Fig (11): Stomach; erosion and ulceration of the glandular region. (H & E, 4x)

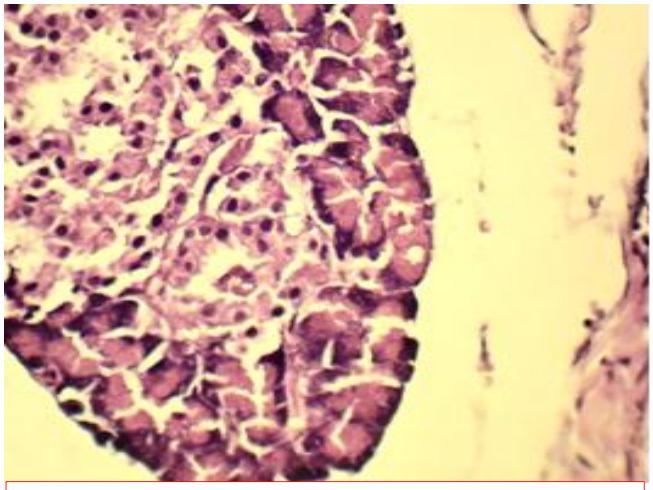
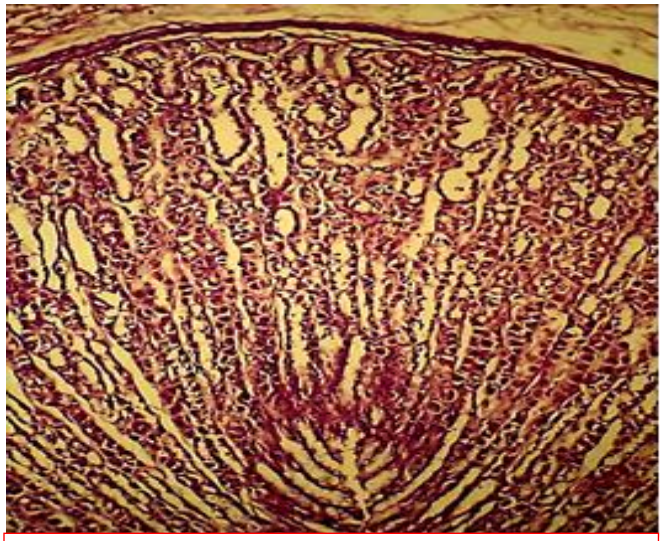


Fig (9): Pancreas; area of vacuolation and degeneration (H & E, 10X)



Fig(12): **Stomach;** (glandular region), vacuolation mucosa and mucous glands. (H & E, 10x)

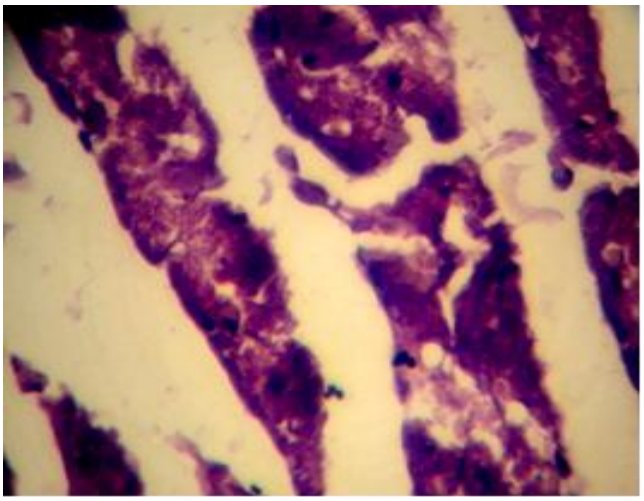


Fig (10): Heart; note vacuolation of myocardial muscle cells. (H & E, 10X)

5. DISCUSSIONS

The non-steroidal anti-inflammatory drugs(NSAIDs) belong to the group of the most abused drugs by virtue of combining the pharmacological actions of anti-inflammatory and analgesia and because they can easily be bought over the counter(Gilman *et al.*, 1990). This study has shown that the NSAIDs when improperly used, could serve as a source of harm to animal organs such as liver, kidney, adrenal gland, pancreas, heart, and stomach. This is because all the NSAIDs used especially Diclofenac Sodium

produced significant increase in the level aspartate aminotransferase (AST) as mentioned by (Bush, 1991; Duncan *et al.*, 1994 and Klaassen, 2001) who reported that Diclofenac Sodium, indomethacin and phenylbutazone caused increase in AST and increase in level of serum alanine aminotransferase (ALP) has been associated with bile duct damage. According to our experiment results; microscopic findings showed prominent changes in the liver such as vacuolation of the hepatocytes sometimes as ballooning like and these agreed with (Smith and Jones, 1986; Hodgson and Levi, 1985 and Klaassen, 2001) who reported that the fact Diclofenac Sodium caused greater damage to the animals is further confirmed by the histopathologic lesions produced. Administration of Diclofenac Sodium caused periportal hepatic necrosis and kupffer cell proliferation. These are all signs of acute hepatoxicity and suggest that Diclofenac Sodium is a hepatotoxicant in rats and the effect on the liver may be one of the causes of death in poisoned rats. On other hand; several research regarded that Diclofenac Sodium inconsequential rather depend on what showed by (Abatan *et al*., 2006 and Lidija *et al*., 2005) who mention that this damage in the liver may not be as serious with Diclofenac Sodium and apart from some degenerative changes in the heart muscle, liver and the kidneys, which are normal at a certain age. The fact that Diclofenac Sodium toxicity showed prominent changes in kidney such as dilated cortical tubules and these result agreed with (Abatan *et al*., 2006) who mention that kidneys of rats showing a focal area of the glomerular and tubular vacuolation and degeneration and presence of protein casts in lumen of tubules.

Other papers mentioned that Diclofenac Sodium showed prominent gastric damage (ulcer) as in (Lichtenberger *et al*., 2007; Naito *et al*., 2001 and Odashima *et al*., 2006) which reported that the Diclofenac Sodium induced gastric ulcer model rats by reduce the gastric juice pH and increase the volume of gastric juice (Wang *et al*., 2007), or decrease the volume of gastric juice and its acid output (Jainu *et al*., 2006). On other hand; several research as (Zhongzhi *et al*., 2011) suggest that the changes in the volume of gastric juice and acid production induced by Diclofenac Sodium are not a major factor in ulcer formation but other research as (Lichtenberger *et al*., 2007 and Wang *et al*., 2007) mention that Diclofenac Sodium has been shown to reduce the mucosal prostaglandins content (PGE2). Prostaglandins have protective effects against various gastric injury models mentioned by (Brzozowski *et al*., 2005and Wallace, 2008). One of the mechanisms by which Diclofenac Sodium damages the gastric mucosa is the increased production of NO due to the overexpression of iNOS (Kontureck *et al*., 2006). NO is a mediator not only of gastrointestinal mucosal defense (Calatayud *et al*., 2001), but also of its damage (Muscara and Wallace, 1999). Other research mentioned that Inflammation and neutrophil infiltration are also important in the pathogenesis of the gastric damage induced by NSAIDs especially aspirin and Diclofenac Sodium (Wallace *et al*., 1990; Lee *et al*., 1992; Trevethick *et al*., 1993; Souza *et al*., 2004). The inflammation induced in the gastric mucosa by Diclofenac Sodium is accompanied by increased

TNF- production (Naito *et al*., 2001; Jainu and Devi, 2006), the levels of TNF were increased by Diclofenac Sodium administration. On other hand; several research regarded that NSAIDs especially aspirin and Diclofenac Sodium reduces colon tumor incidence in rats showed by (Denis and Fabrice, 2005) who mention that analysis of subsets where Diclofenac Sodium was given only before or after the initiation is compatible with the hypothesis that the protection is higher when aspirin treatment is given during initiation tumor in rats. Other papers mentioned that NSAIDs especially aspirin and Diclofenac Sodium treatment did not only reduce the number of colonic polyps as mentioned by (Perkins *et al*. 2003) which explain that the aspirin prevents the early phase of carcinogenesis, and would be active before birth and until weaning.

Conclusions

The toxological pathology of Diclofenac Sodium in laboratory white rats showed:

1. Treatment related changes in the liver mainly as varying degrees of vacuolation in the centrolobulare region and septal fibrosis.
2. Toxologic pathology lesions in the kidney as dilated cortical tubules.
3. Toxologic pathology changes in the islet of Langerhans of pancreas mainly as vacuolation.
4. Degeneration and vacuolation of myocardial muscles cells of the heart.
5. Vacuolation of zona vasculata of adrenal glands.
6. Erosion / ulceration of the glandular region of the stomach.

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