Toxic - Pathological Study for Long – Term Administration of Aspirin in White Rats

دراسة سمية ـ إمراضيه لتعاطي الاسبرين طويل الامد في الجرذان البيضاء

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Abstract

The study showed that the starting dose 200 mg/kg for two months then increasing the dose to 400 mg/kg for another two months and finally the last two months the dose was 600 mg/kg. during the study no clinical sings were seen and none of the treated animals died during the experiment. This indicate tolerance of the rat to toxicity of the aspirin. Only histopathological changes were seen after termination and those were in the liver, kidney, heart, pancreas and stomach. These were as follows, vaculation and degeneration of liver cells, dilated cortical tubules in kidney, degeneration and vaculation of islet of pancreas, vaculation of myocardial muscle cells in the heart and erosion and ulceration of the glandular stomach.

الخلاصة

اظهرت الدراسة بأن بداية الجرعة 200 ملغ/كغم لمدة شهرين من بدء التجربة ثم نرفع مستوى الجرعة الى 400 ملغ/كغم للشهرين اللاحقين وفي الشهرين الاخيرين من التجربة نعطي 600 ملغ/كغم من الاسبرين.
خلال الدراسة لم نشاهد علامات سريريه ولم ينفق أي حيوان من المجموعة المعاملة بالمادة العلاجية طيلة فترة التجربة وهذا يشير الى قابلية تحمل الجرذان لسمية الاسبرين.
شوهدت فقط تغيرات نسيجيه مرضيه بعد نهاية فترة التجربة والتي كانت واضحة في الكبد والكلية والقلب وغدة البنكرياس والمعدة وهذه كانت كالتالي، تجوف و تنكس الخلايا الكبدية وتوسع النبيبات الكلوية وتنكس وتجوف جزر لانجرهانز في البنكرياس وتهتك وتقرح بطانة المعدة الغدية.

Introduction

Non-steroidalanti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, naproxen, and indomethacin.

Aspirin is a potent nonsteroidal anti-inflammatory drug (NSAID) that is used for the treatment of rheumatoid arthritis and related diseases as well as the prevention of cardiovascular thrombotic diseases. Gastric ulcer associated with the use of aspirin 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).

Aspirin is extensively used as analgesics and anti-inflammatory agents and produce therapeutic effect through the inhibition of prostaglandin synthesis (Klaassen, 2001). Aspirin 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, aspirin 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).

Aims of the study:

i. To study the toxicological pathology of aspirin by using white rats as experimental model.

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

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

Materials and Methods
Animals and Design:

White rats of both sexes weighing between 200-250 gm were used for the experiment. They were separated into 2 groups (control and treat), each group consisting of 4 animals, 2 males and 2 females. Then be subjected from October to march 2011. Animals were housed in wire mesh cages under ambient light conditions, with adding aspirin to fresh water into treat group. They were acclimatized to captivity for at least two weeks prior to testing. Aspirin (Vardnman Export, India) at

200 mg/kg body weight; for two months then increasing the dose to 400 mg/kg for another two months and finally the last two months the dose was 600 mg/kg. The drug were dissolved in distilled water before administration by dose to each animal in the group orally using a stomach cannula for six months. The animals were observed in their cages for clinical symptoms daily. After six 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.

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).

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 aspirin

Results

Results of our 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 toxologic pathology changes were in the liver and those changes were varied from vaculation of hepatocytes / ballooning like with interstitial edema (figs 1,2). On occasion those changes were associated with advanced degrees of septal fibrosis (figs 3,4) also there were centrilobular vaculation of hepatocytes (figs 5,6).

Furthermore, other toxologic pathologic changes in other visceral organs and tissues were varied from varying degrees of dilatation of renal cortical tubules (figs 7,8) and other changes associated with vaculation of zona fasculata and reduce size of the adrenal gland (figs 9,10) In addition further histopathological changes in pancreas as degeneration and vaculation of islet of langerhans (figs 11,12) while in heart it was vaculation of myocardial muscle cells (figs 13,14), other important histopathological changes were vaculation, erosion and ulceration in the glandular region of stomach(figs 15,16).

The toxologic pathology study of aspirin in rats showed that, the rats is highly tolerant to aspirin as during the experiment gradually increase of the dose from 200 mg / kg for the first two months, 400 mg/ kg for the next two months and finally 600 mg / kg for the last two months. 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.
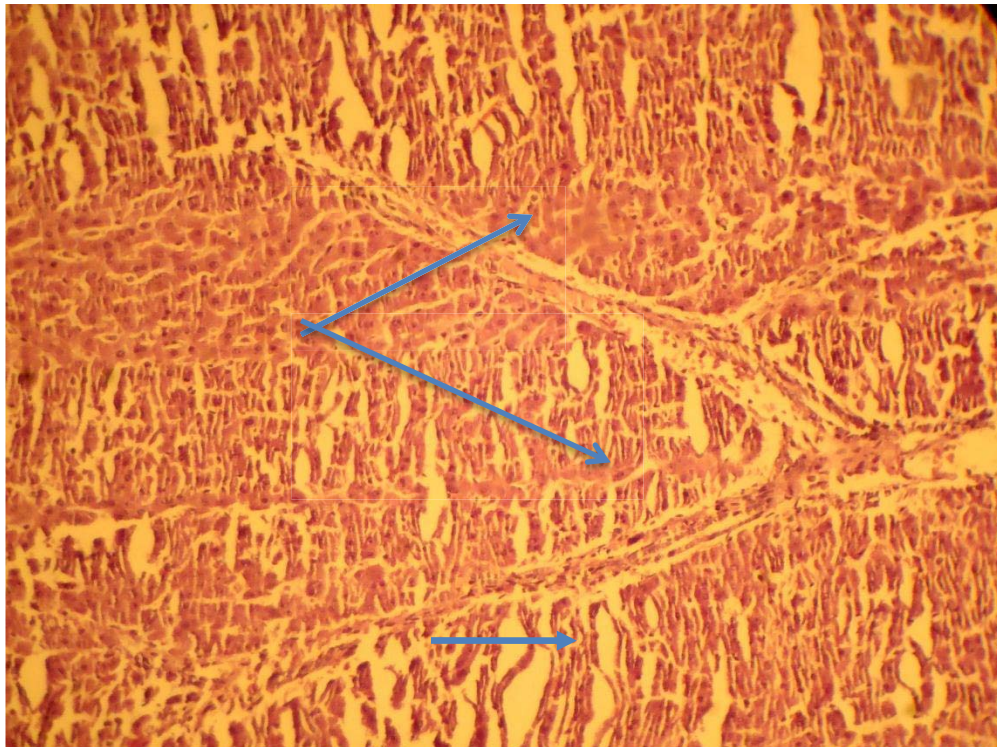
Note: all sections were taken transversely or obliquely from visceral organs of both male and female.
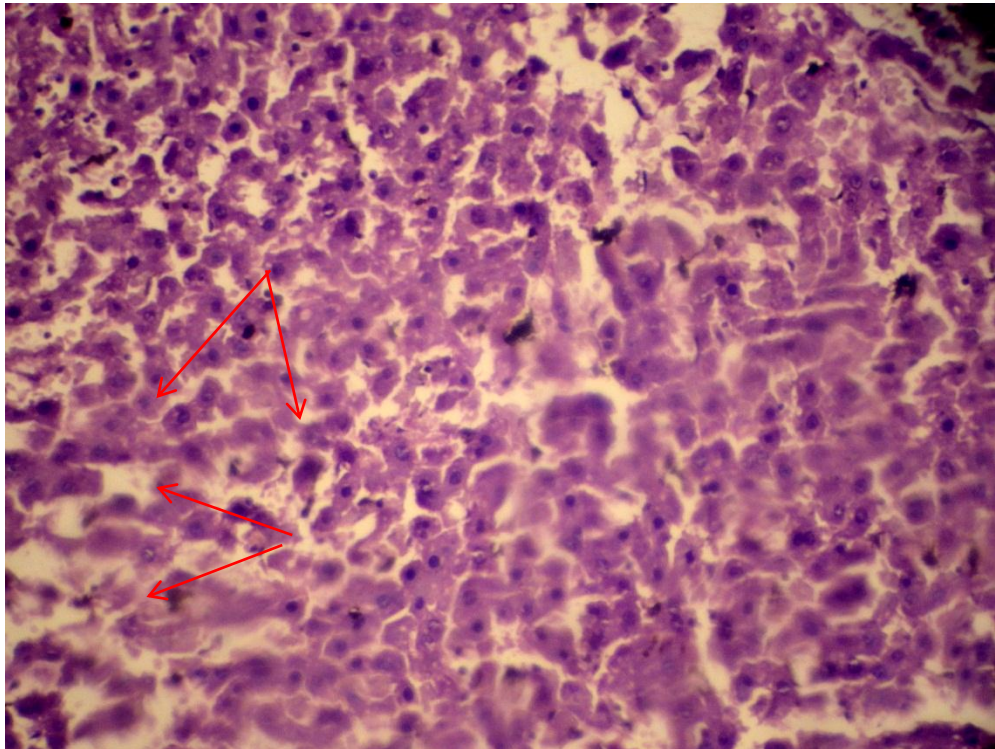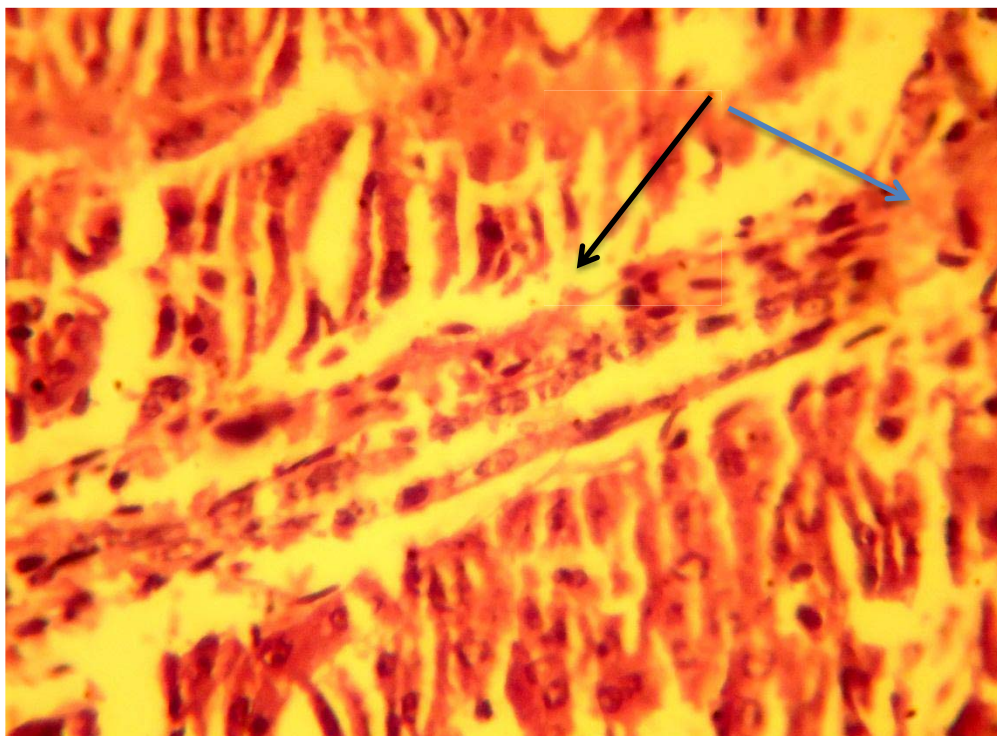

Fig(1): Liver ; an area of marked vaculation / ballooning like of hepatocyes.
       (H & E, 10X)
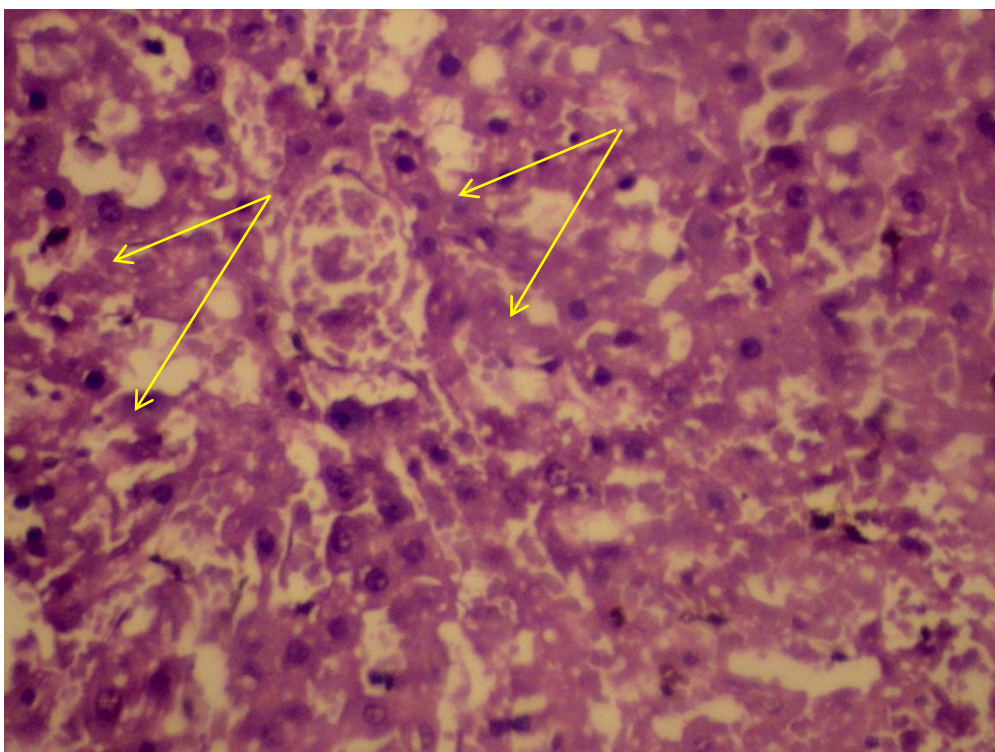
Fig(2): Liver; degeneration and vaculation of hepatocytes.
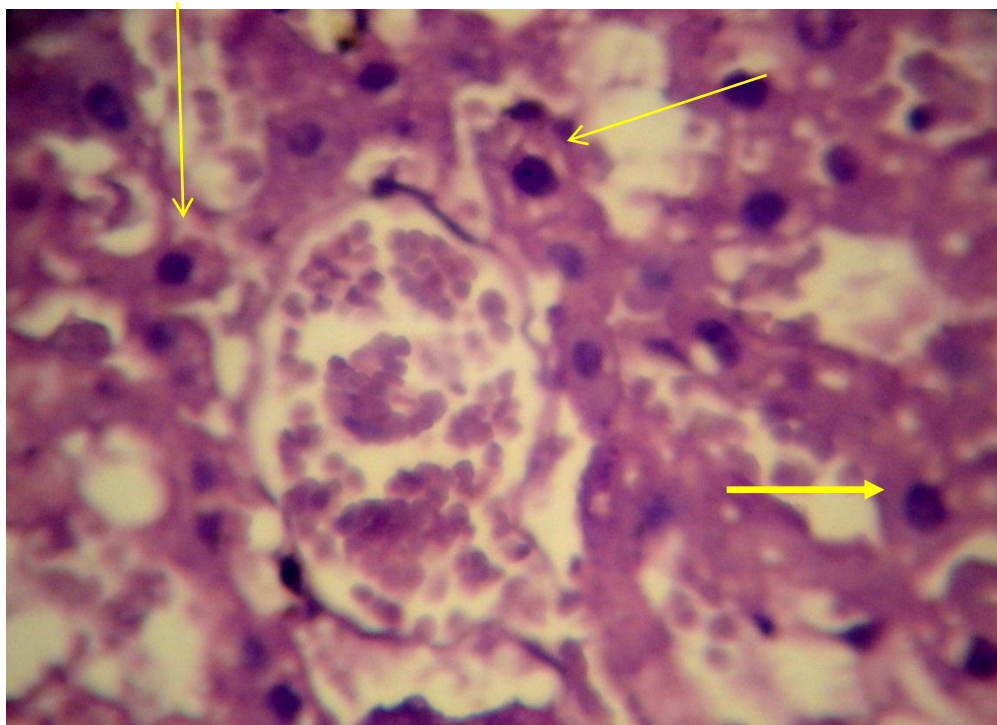
   (H & E, 40X)

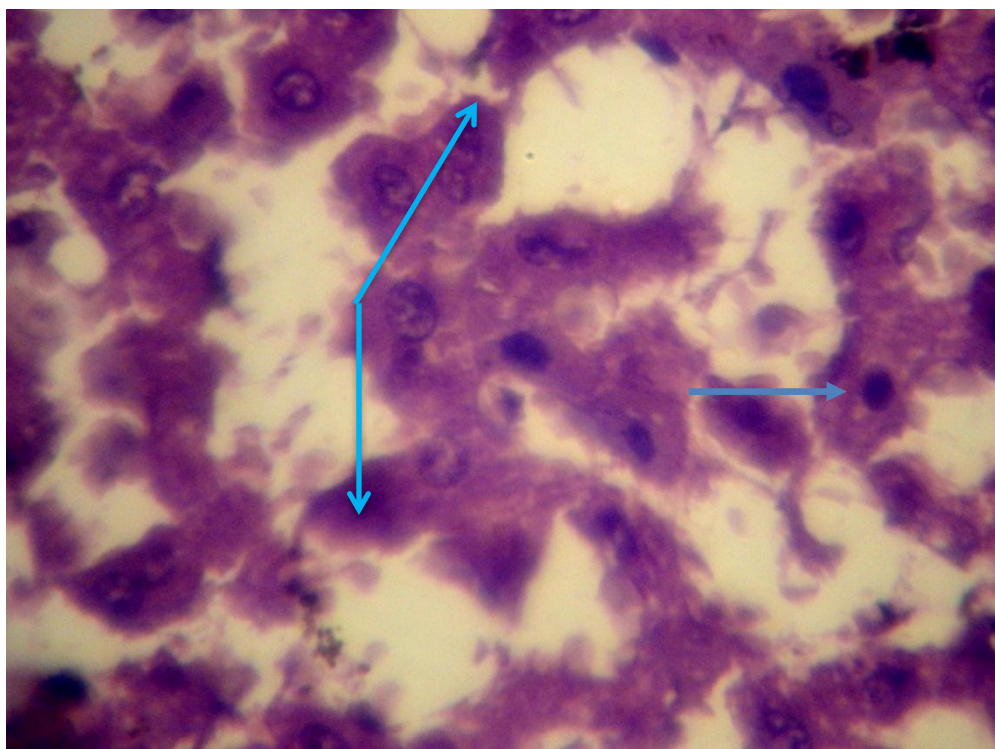**Fig(3): Liver; septal fibrosis.   (H & E, 10X)**

Fig(4): Liver; septal fibrosis   with interstitial edema   (H & E, 40X)
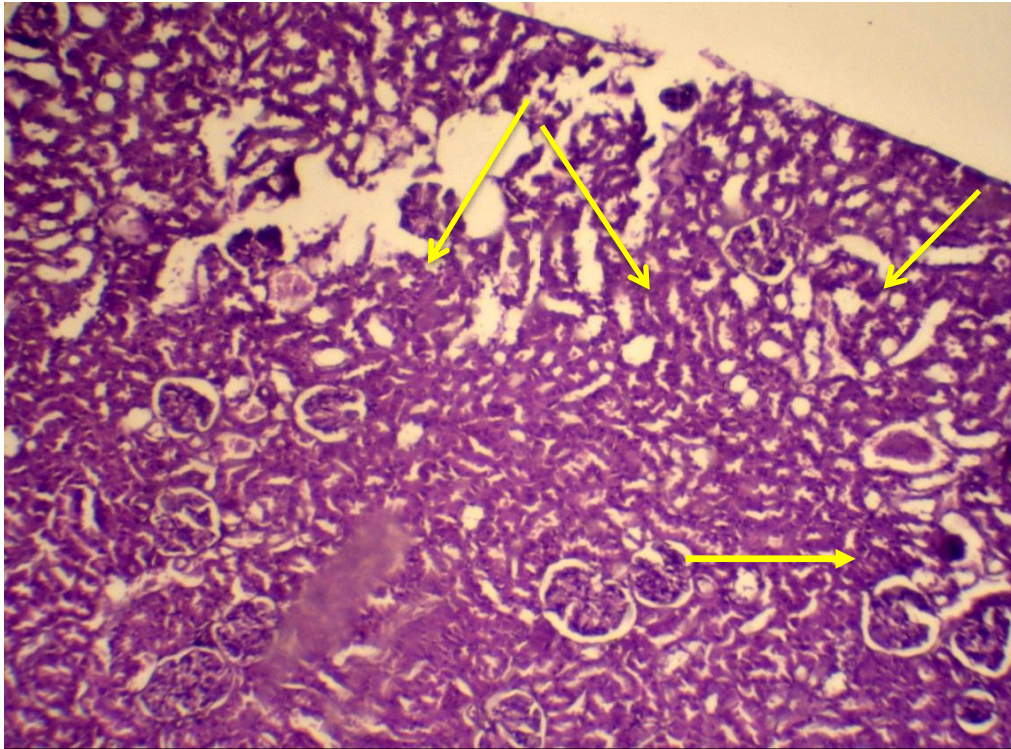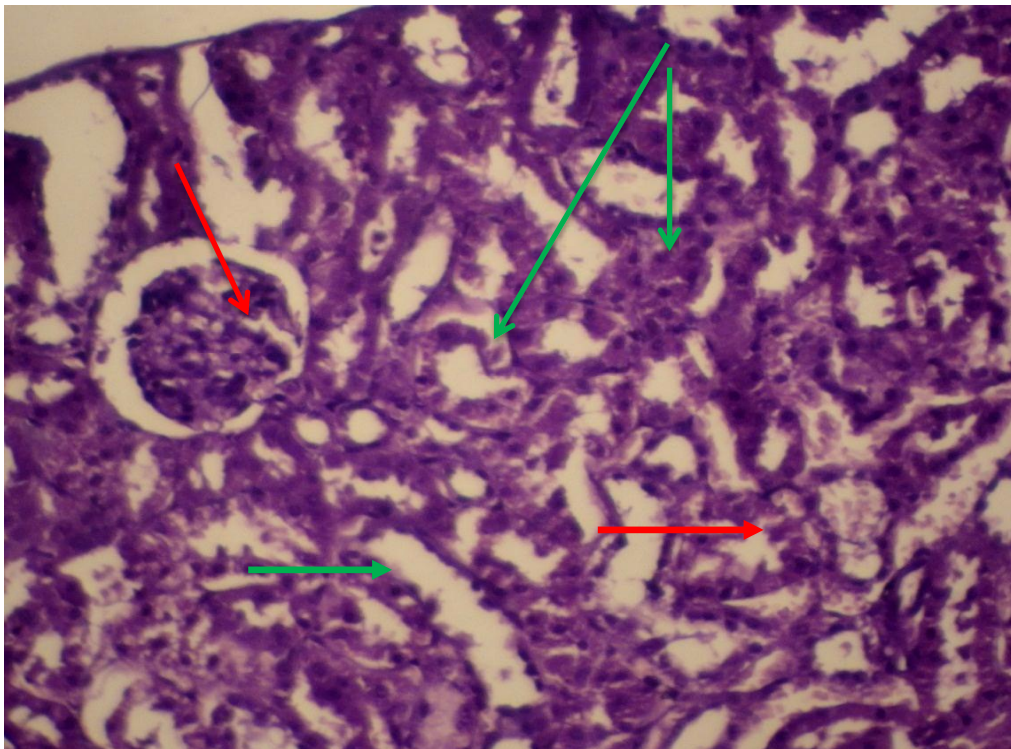
Fig(5): Liver; centrolobular vaculation of hepatocytes.   (H & E, 10X)
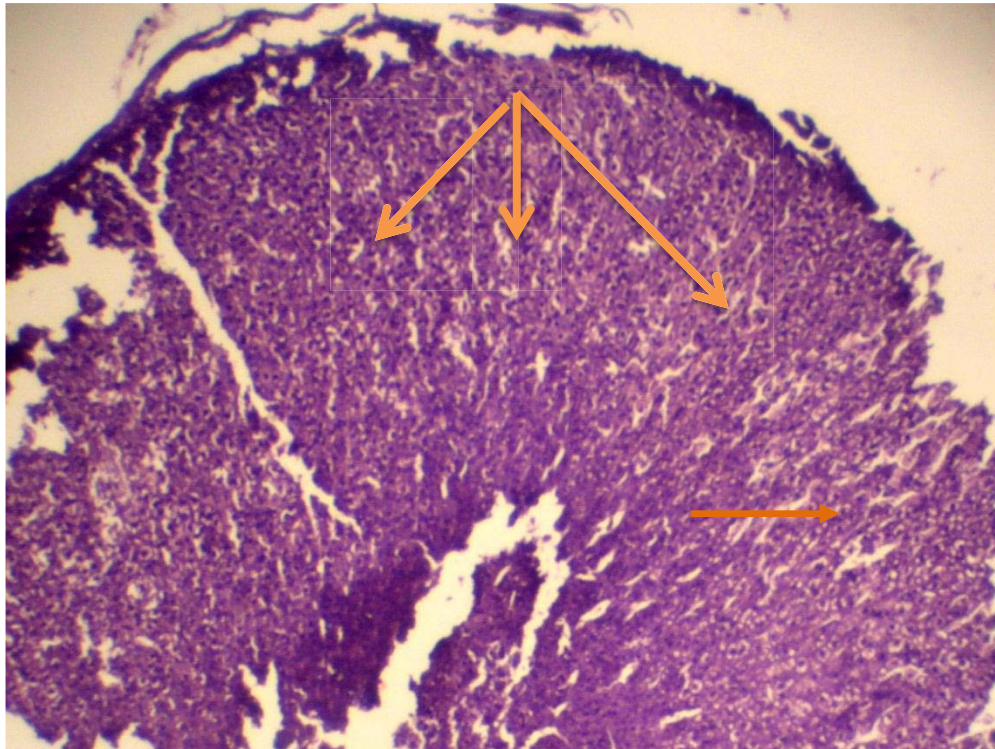

Fig(6): Liver; centrolobular vaculation of hepatocytes. (H & E, 40X)
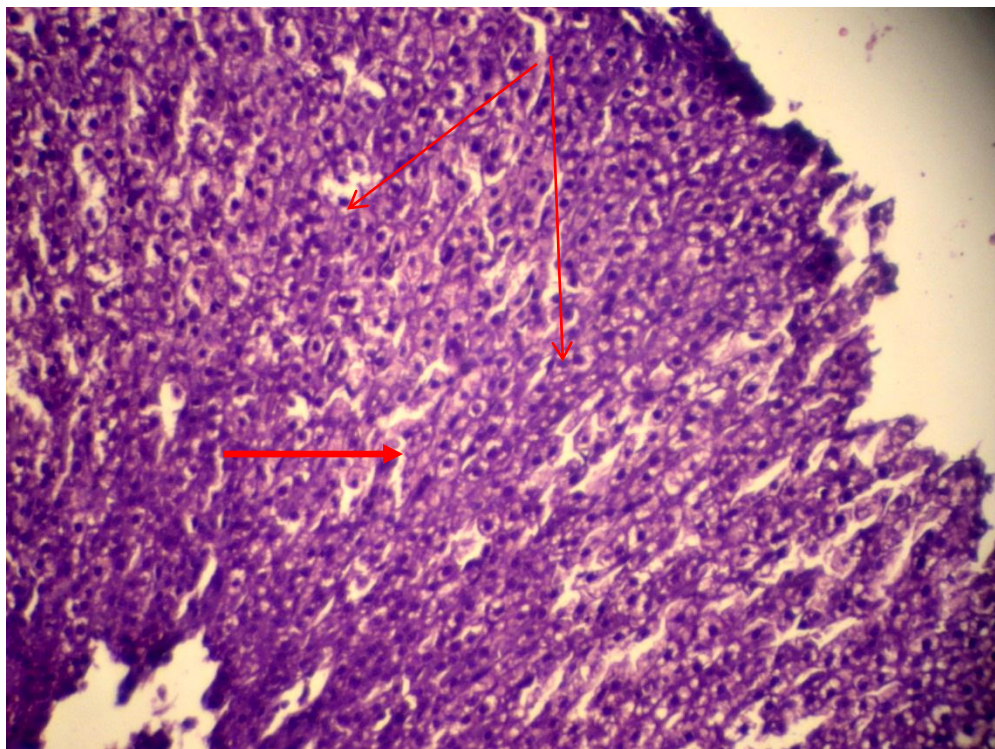
Fig(7): Kidney; marked dilation of cortical tubules. (H & E, 10X)

Fig(8): Kidney; note vaculation of glomeruli   and area of dilation of cortical tubules. (H & E, 40X)

Fig(9): Adrenal gland; vaculation of zona vasculata and reduce in size. (H & E, 4X)

Fig(10): Adrenal gland; vaculation and degeneration of zona vasculata and reduce in size. (H & E, 10X)
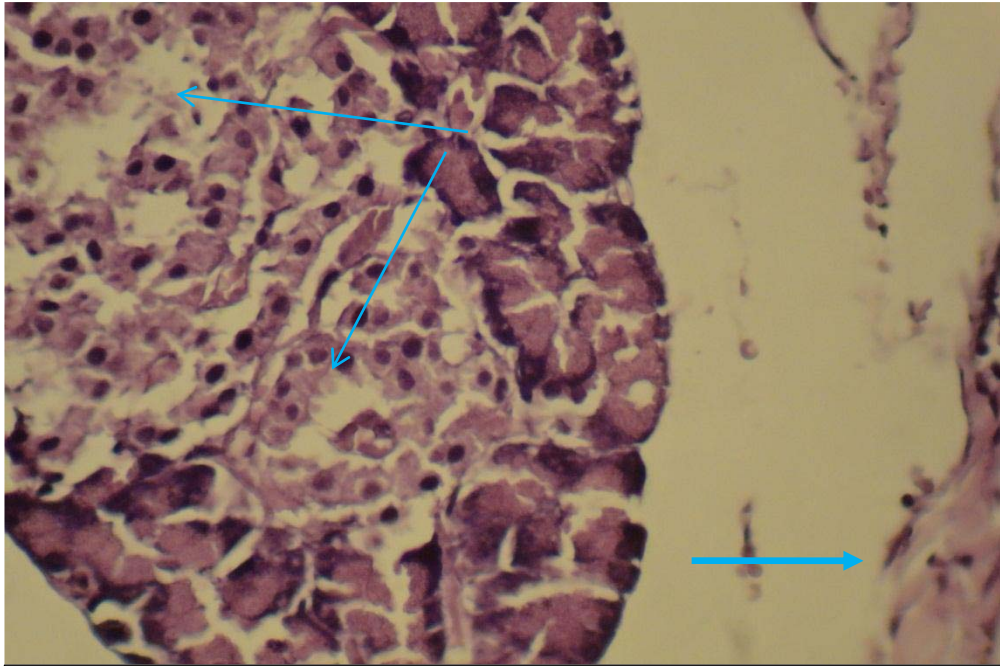
Fig(11): Pancreas; area of vaculation and degeneration.   (H & E, 10X)
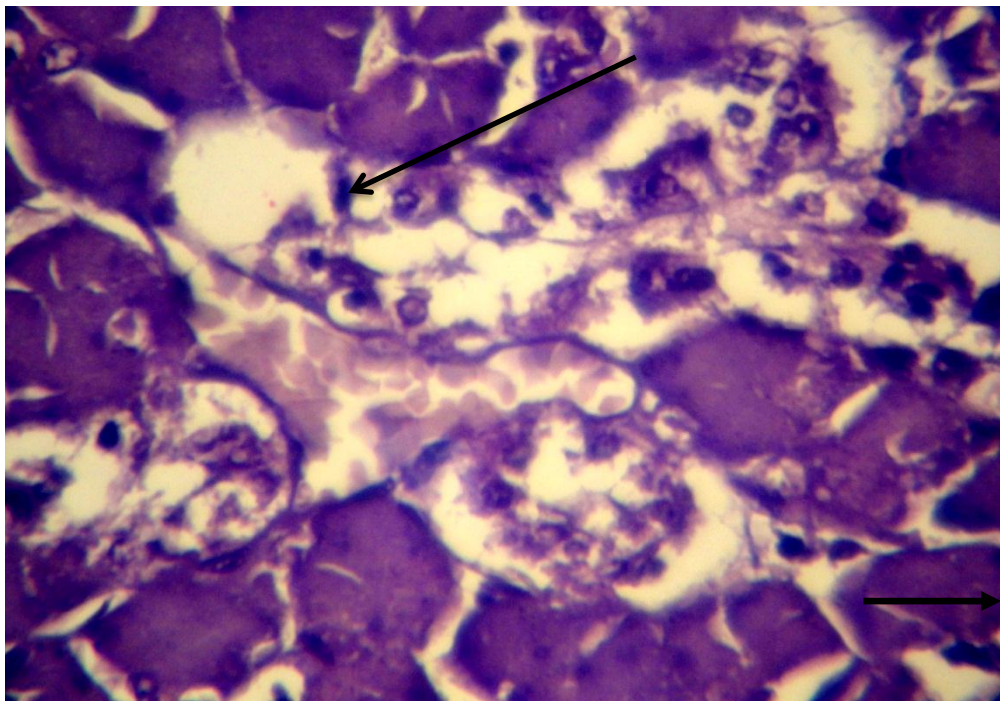
**Fig(12): Pancreas; vaculation and degeneration of islet of langerhans.
(H & E, 40X)**
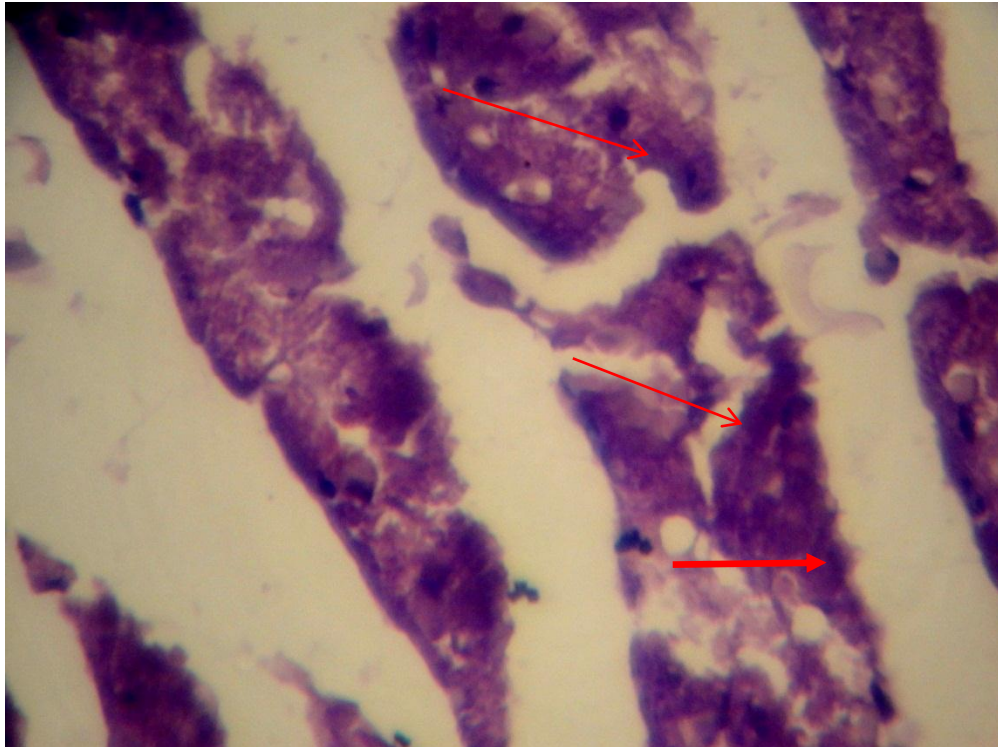
Fig(13): Heart; note vaculation of myocardial muscle cells.   (H & E, 10X)
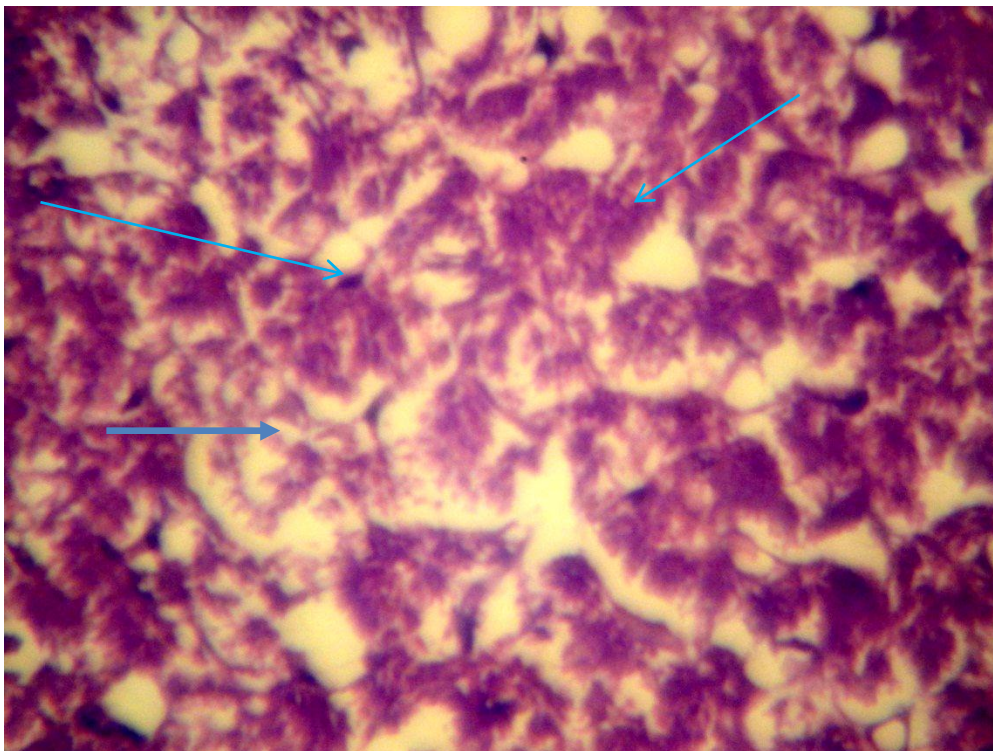
Fig(14): Heart; intercellular edema with vaculation of myocardial muscle cells.   (H & E, 40x)

Fig(15): Stomach; erosion and ulceration of the glandular region. (H & E, 4x)

Fig(15): Stomach; (glandular region), vaculation mucosa and mucous glands.   (H & E, 10x)

Discussion

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).

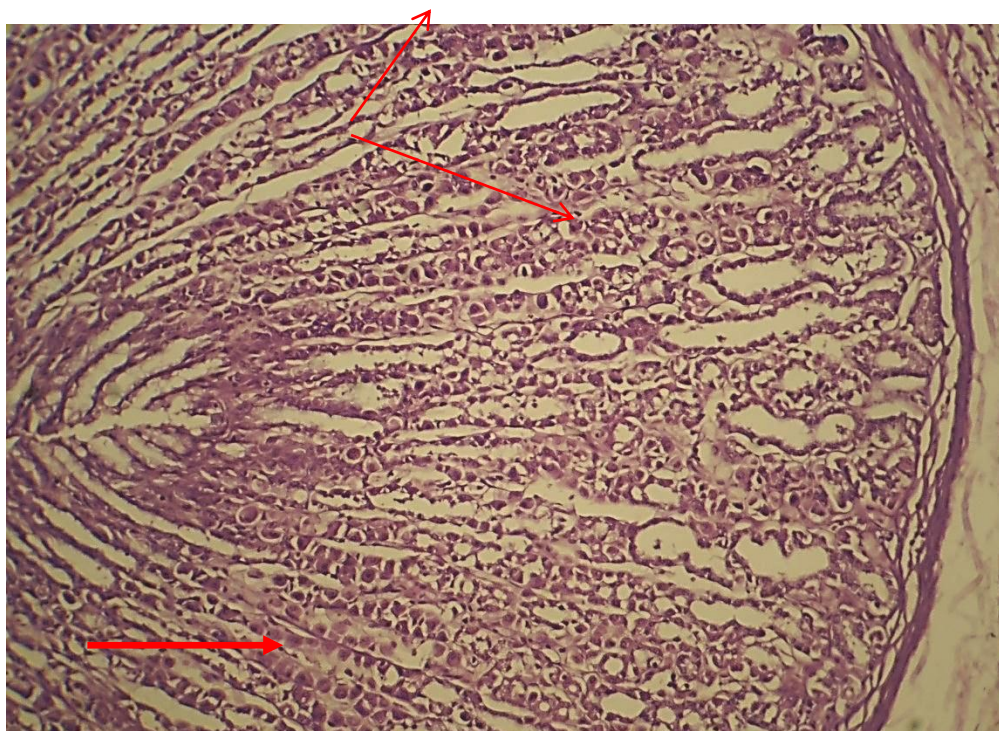This study has shown that the NSAIDs when improperly used, could serve as a source of harm to animal organs such as liver, kidney, adrenal gland, pancreas, heart, and stomach. This is because all the NSAIDs used especially aspirin produced significant increase in the level aspartate aminotransferase (AST) as mentioned by (Bush, 1991; Duncan *et al.,* 1994 and Klaassen, 2001) who reported that aspirin, 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 vaculation 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 aspirin caused greater damage to the animals is further confirmed by the histopathologic lesions produced. Administration of aspirin caused periportal hepatic necrosis and kupffer cell proliferation. These are all signs of acute hepatoxicity and suggest that aspirin 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 aspirin 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 aspirin and apart from some degenerative changes in the heart muscle, liver and the kidneys, which are normal at a certain age.

The fact that aspirin 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 vaculation and degeneration and presence of protein casts in lumen of tubules.

Other papers mentioned that aspirin showed prominent gastric damage (ulcer) as in (Lichtenberger *et al*., 2007; Naito *et al*., 2001 and Odashima *et al*., 2006) which reported that the Aspirin 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 aspirin are not a major factor in ulcer formation but other research as (Lichtenberger *et al*., 2007 and Wang *et al*., 2007) mention that Aspirin 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 aspirin 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)3.

Other research mentioned that Inflammation and neutrophil infiltration are also important in the pathogenesis of the gastric damage induced by NSAIDs especially aspirin (Wallace *et al*., 1990; Lee *et al*., 1992; Trevethick *et al*., 1993; Souza *et al*., 2004). The inflammation induced in the gastric mucosa by aspirin is accompanied by increased TNF- production (Naito *et al*., 2001; Jainu and Devi, 2006), the levels of TNF were increased by aspirin administration.

On other hand; several research regarded that aspirin reduces colon tumor incidence in rats showed by (Denis and Fabrice, 2005) who mention that analysis of subsets where aspirin 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 aspirin 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 aspirin in laboratory white rats showed:

1. Treatment related changes in the liver mainly as varying degrees of vaculation 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 vaculation.
4. Degeneration and vaculation of myocardial muscles cells of the heart.
5. Vaculation of zona vasculata of adrenal glands.
6. Erosion / ulceration of the glandular region of the stomach.

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