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Histological Studies on The Effect of Diclofenac Sodium on The Foetal Pancreas of Albino Mice

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ABSTRACT

Background: Diclofenac sodium (DS) is regarded as one of the most significant and often used medications. Numerous cases of rheumatoid arthritis, osteoarthritis and soft tissue rheumatism are all treated with it. Despite the drug's positive effects, some medical studies accuse it of having pathogenic effects after being used for medicinal purposes on the body's organs.

Objective: The purpose of the current research was to assess the histological impact of the non-steroidal anti-inflammatory drug, diclofenac sodium (DS) on the pancreas of albino mice foetuses.

Material and methods: Six groups of 60 pregnant female mice were prepared (10 mice each). Each animal in the first and second groups received an intraperitoneal (IP) injection of the drug's solvent every day for six days, from day 1 till day 6 of gestation (GDs 1-6) and from day 7 to day 14 of gestation (GDs 7–14), respectively. These groups served as the control groups. The third and five groups are the treated groups, receiving daily (IP) injections of 1.5 and 3 mg/kg body weight of DS from days 1 through 6 of gestation (GDs 1-6), respectively, whereas the animals in groups fourth and six received similarly daily injections of 1.5 and 3 mg/kg body weight of DS for 8 days (GDs 7-14), respectively.

Results: The pancreas of maternally treated foetuses underwent histological study, which revealed signs of alteration in both the exocrine and endocrine structures. These features varied according to the dose and period of administration, and they included focal acinar damage that manifested as cytoplasmic vacuolation and necrosis, pyknotic, and karyolysed nuclei, as well as blood extravasations that revealed hemorrhagic appearances.

Conclusion: Because of the damaging effects of DS on the developing pancreas of mice, it should only be administered under rigorous control in the medical profession to safeguard expectant mothers from its potentially harmful effects.

Keywords: Diclofenac sodium, Foetuses, Histopathology, Mice, Pancreas, Implantation, Organogenesis.

INTRODUCTION

Non-steroidal anti-inflammatory medicine (NSAID) diclofenac (DCLF) is widely prescribed to treat osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and sudden onset of muscle pain. A well-known mechanism of action for NSAIDs is the inhibition of cyclooxygenases, the rate-limiting enzymes that catalyse the generation of prostaglandin precursors from arachidonic acid [1, 2].

Prostaglandins are involved in the modulation of immunological responses and the regulation of cell proliferation [3, 4]. Despite their helpful benefits, several medical reports have indicated the toxic and pathological side effects of these medications on the liver, kidneys, and gastrointestinal system, among several other body organs [4].

In a communication offered by **Bombardo *et al.*** [5] after inducing pancreatitis in C57BL/6 mice with successive cerulein injections, the NSAIDs ibuprofen and diclofenac prevent the proliferation of pancreatic acinar cells, and the authors speculated that long-term use of these NSAIDs may harm the pancreas' ability to regenerate. In line with this theory, ibuprofen in the absence of cancer-related diseases, slows down the advancement of the cell cycle through the G1 phase [6].

Importantly, this extended lifetime was preserved across several species, including in yeast cells devoid of COX enzymes [7]. Additional research also showed that ibuprofen inhibited the development of non-cancerous cells, such as endothelial cells and smooth muscle cells in the human coronary arteries [8, 9].

Although NSAID consumption has increased despite these new findings, even short-term use of NSAIDs during the late stages of pregnancy is significantly linked to an increased risk of premature closure of the ductus arteriosus [10]. It is important to note that selective COX-2 inhibitors, which are frequently recommended during pregnancy, might enter the placenta even when the baby does not consume them [11]. There has been a great deal of research done on the biochemical and histological effects of NSAIDs on the pancreas of adult experimental animals. The impact of diclofenac sodium (DS) on the pancreatic tissue of the developing foetuses, there are, however, limited data that are now accessible. The aim of the current investigation was to examine potential histological changes in the pancreas of mice foetuses whose mothers received DS treatment during the implantation and organogenesis phases of pregnancy.

MATERIALS AND METHODS

Experimental animals

Theodor Bilharz Research Institute (TBRI), Imbaba, Giza, A.R. Egypt's breeding unit provided the

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adult albino mice used in this study, which had a body weight of 20 to 30g on average. In order to prevent overcrowding, females and male mice were kept apart in plastic cages, each of which held two mice. Mice were given cubes made of unprocessed proteins, minerals, and fibres. The animals were given unlimited access to milk and tap water *ad libitum*, as well as fresh vegetables that supplied vitamins. One adult female was kept overnight with an adult male from 5 p.m. to 9 a.m. the following day, to become pregnant. According to **Snell's** [12] approach, a vaginal plug or spermatozoa in the vaginal smears were signs of a successful mating. The first day of pregnancy was defined as the day on which a female provided a positive vaginal smear, and these mothers are deemed to be pregnant.

The drug used

Diclofenac sodium (DS) was the medication employed in the current investigation (Declophen; Pharco Pharmaceutical Company, Egypt). The selected doses of diclofenac sodium were almost identical to the effective therapeutic doses for humans. According to **Paget and Barnes** [13]**,** the chosen dosages of the medication for mice were determined to be comparable to 1.5–3 mg/Kg body weight.

Experimental design

A group of 60 pregnant female mice were divided into six groups (10 mice each). The first two groups were the control groups (C_1 & C_2) and the last four groups (A, B, D & E) were the drug treated groups and treatments of these four groups were achieved in the following manner:

Group (C_1): Each pregnant female was injected intraperitoneally (IP) with 0.1ml distilled water (the solvent of the drug) daily for 6 days during pregnancy from day 1 till day 6 of gestation; gestation days (GDs 1-6).

Group (C_2): Each pregnant female was injected IP with 0.1ml distilled water daily for 8 days during pregnancy, from day 7 till day 14 of gestation (GDs 7-14)**.**

Group (**A):** Each pregnant female was injected IP with 1.5mg/kg body weight of diclofenac sodium for 6 days during pregnancy, from day 1 till day 6 of gestation (GDs 1-6).

Group (**B):** Each pregnant female was injected IP with 1.5mg/kg body weight of diclofenac sodium for 8 days during pregnancy, from day 7 till day 14 of gestation (GDs 7-14).

Group (**D):** Each pregnant female was injected IP with 3mg/kg body weight of diclofenac sodium for 6 days during pregnancy, from day 1 till day 6 of gestation (GDs 1-6).

Group (**E):** Each pregnant female was injected IP with 3mg/kg body weight of diclofenac sodium for 8 days during pregnancy, from day 7 till day 14 of gestation (GDs 7-14).

Pregnant mice from the control and experimental groups were killed on day 19 of pregnancy. They were dissected, had their uteri removed, were placed in a regular saline solution, and the foetuses were extracted. Small pancreatic organs from untreated and maternally treated animals' foetuses were preserved in aqueous Bouin's fixative for 24 hours to be examined under a light microscope. The samples were dehydrated, cleaned with terpineol, and then set with paraffin wax. Serial transverse slices around 5µm thick were made and stained with haematoxylin and eosin, then inspected under a microscope, and given a photomicrograph.

RESULTS

Histological and histopathological observations

I- The control mice foetuses

The foetuses of control mice have a thin connective tissue capsule covering their pancreas, and septa are sent from this capsule dividing it into lobules. An intricate coating of reticular fibres surrounds and supports the acini. The capillary network is quite dense. Exocrine and endocrine glands coexist in the pancreas.

The exocrine component is made up of acinar cells, which have granules at the tip of their pyramidal structure. Centro-acinar cells, which make up the intra-acinar component of the intercalated duct, are formed when the intercalated duct partially enters the acini. The islets of Langerhans are scattered haphazardly throughout its exocrine region. The islets include varying quantities of endocrine cells. Each islet is enclosed by a delicate reticular fibre capsule and is made up of cords of polygonal or rounded cells that are stained by hematoxylin and eosin less deeply than the pancreatic acinar cells (Fig. 1, a-d). There are three different sorts of cells, but a light microscope has a hard time telling them apart.

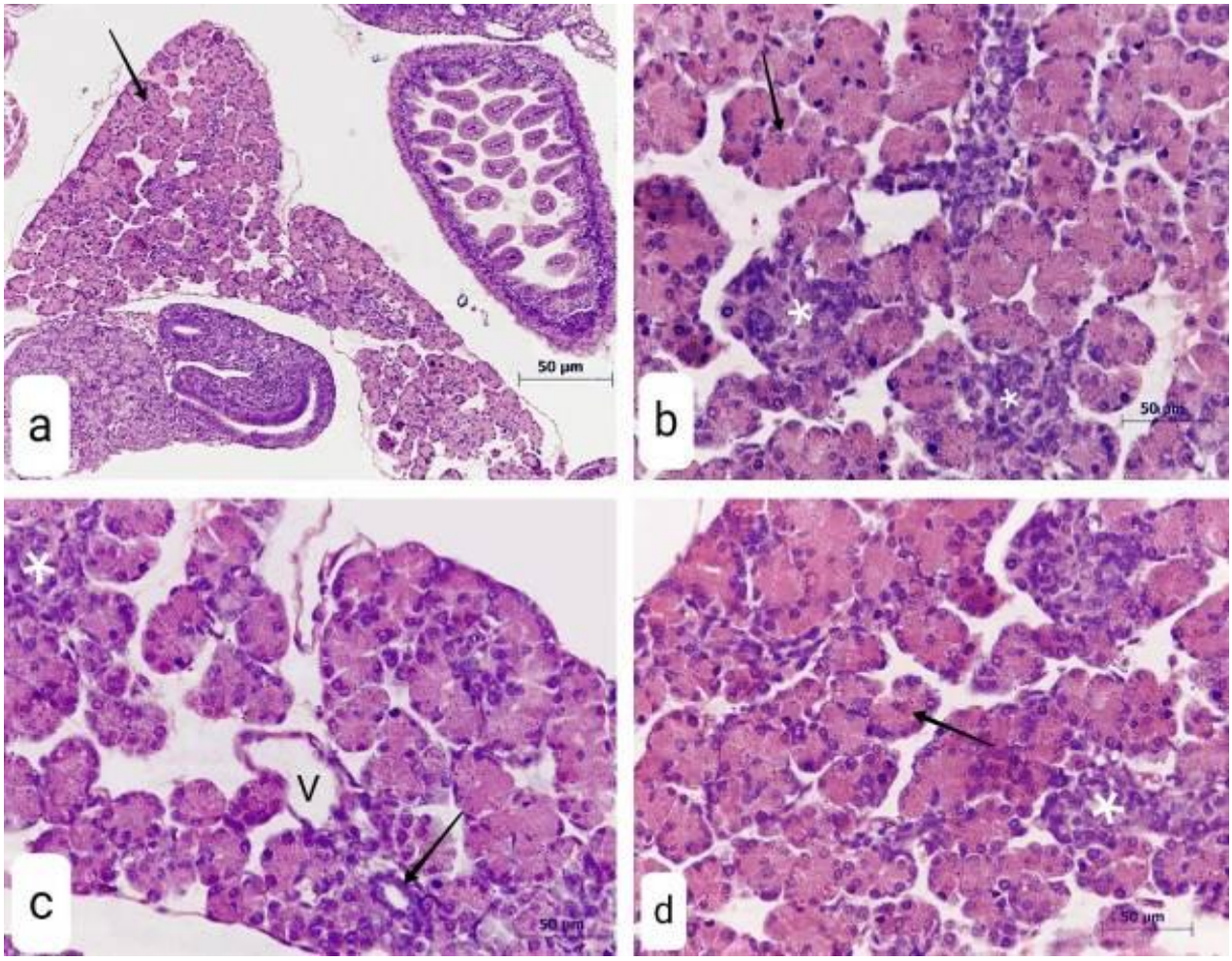

Figure (1): Photomicrographs of pancreas sections of 19-days old control mice foetus illustrating: **a**-whole pancreas (arrow) surrounded by sections of other organs. **b**- compactly arranged lobules of healthy pancreatic acini and islets (*), pyramidal cells with basal nuclei and acidophilic cytoplasm make up the acini (arrow). **c**- pancreatic acini with Langerhans islets (*), pancreatic vein (V) and pancreatic ductulus (arrow). **d**- pancreatic acini (arrow), with Langerhans islets (*).

II - Group A (1.5 mg/kg body weight DS maternally treated foetuses; GDs 1-6)

The pancreas of 19-days old mice foetuses maternally treated with 1.5mg/kg body weight of DS during pre-implantation and implantation periods exhibited signs of alterations in both exocrine and endocrine parts of the pancreas. The pancreatic acinar cells revealed focal acinar damage appeared in a form of cytoplasmic vacuolation with pyknotic and karyolysed nuclei in addition to extravasations of blood from blood vessels forming hemorrhagic appearance (Fig. 2, b). The endocrine part appeared to be irregular in shape. (Fig.2).

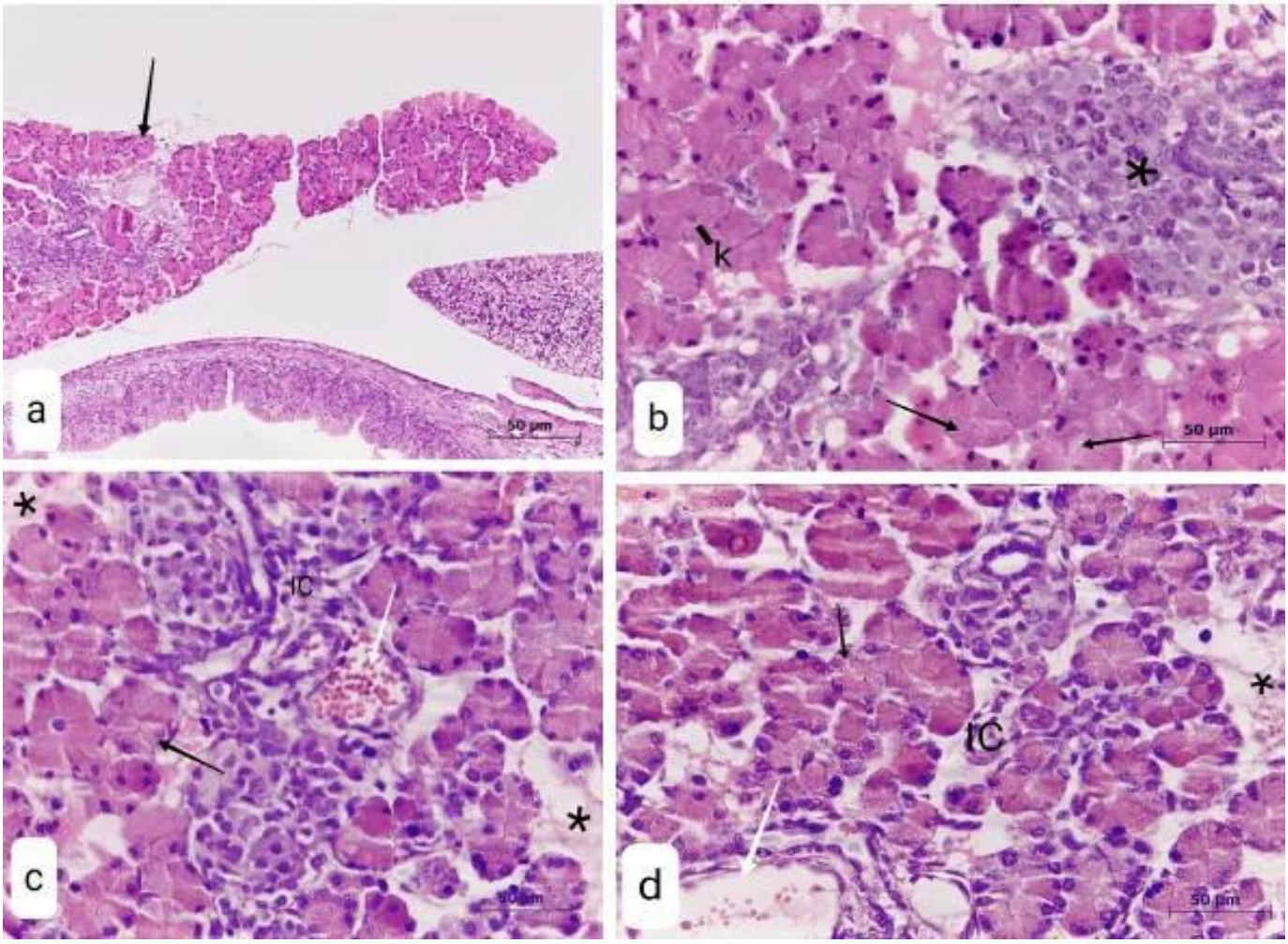

Figure (2): Photomicrographs of pancreas sections of 19-days old mice foetuses maternally treated with 1.5 mg/kg of DS during pre-implantation &implantation period; GDs (1-6) showing: a-whole affected pancreas (arrow). **b-** pancreatic acini with cytoplasmic vacuolations (arrows); their nuclei exhibit marked karyolysis (K). Irregularity in islets of Langerhans (*) was also observed. **c & d-** vacuolar degeneration of the acini components (arrow) with focal aggregation of inflammatory cells (IC) and marked bleeding (*) are noticed between the connective tissue of the supporting pancreatic lobules with congestion of blood vessels (white arrow).

III - Group B (1.5 mg/kg body weight DS maternally treated foetuses; GDs 7-14)

During organogenesis, the pancreas of 19-day-old mouse foetuses maternally treated with 1.5 mg/kg body weight of DS showed evidence of changes in pancreatic tissues. Degenerative alternations to the acini's lining epithelial cells were among these changes (Fig. 3). These acinar cells have vacuolated cytoplasm and karyolyzed nuclei (Fig. 3). Other cells underwent necrosis, lost their normal architecture, and had characteristic pyknosis and karyolysis features in their nuclei. Blood extravasations showing oedema, congested and dilated blood vessels, were also seen (Fig.3).

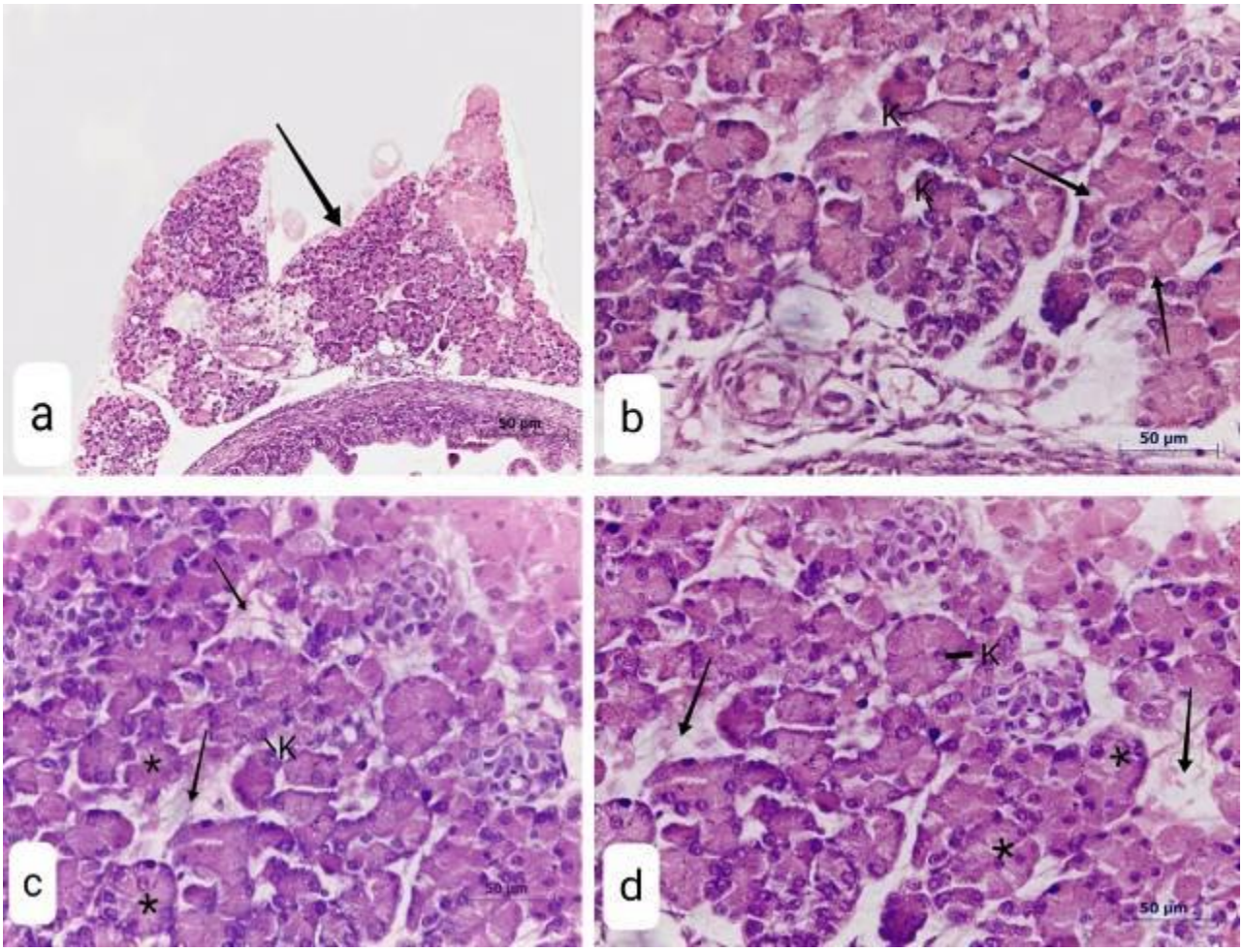

Figure (3): Photomicrographs of pancreas sections of 19-days old mice foetuses maternally treated with 1.5 mg/kg of DS during organogenesis; GDs (7-14) illustrating. a- whole affected pancreas (arrow) with stomach section. **b-** pancreatic acinar cells with vacuolated cytoplasm (arrows) and karyolysed (K) nuclei. **c & d-** loss of the normal architecture of lobules, acinar cells with vacuolated cytoplasm (*) and nuclei with karyolysis (K) features. Blood extravasations forming oedema (arrows) with congested and dilated blood vessels.

IV - Group D (3 mg/kg body weight DS maternally treated foetuses; GDs 1-6)

During the pre-implantation and implantation periods, the pancreas of 19-day-old mouse foetuses maternally treated with 3mg/kg body weight of DS showed severe symptoms of changes in pancreatic tissues. The normal architecture of the acini was lost, and these acini also illustrated necrosis, interstitial haemorrhage, and ductal dilatation as a result of the blood artery damage (Fig.4).

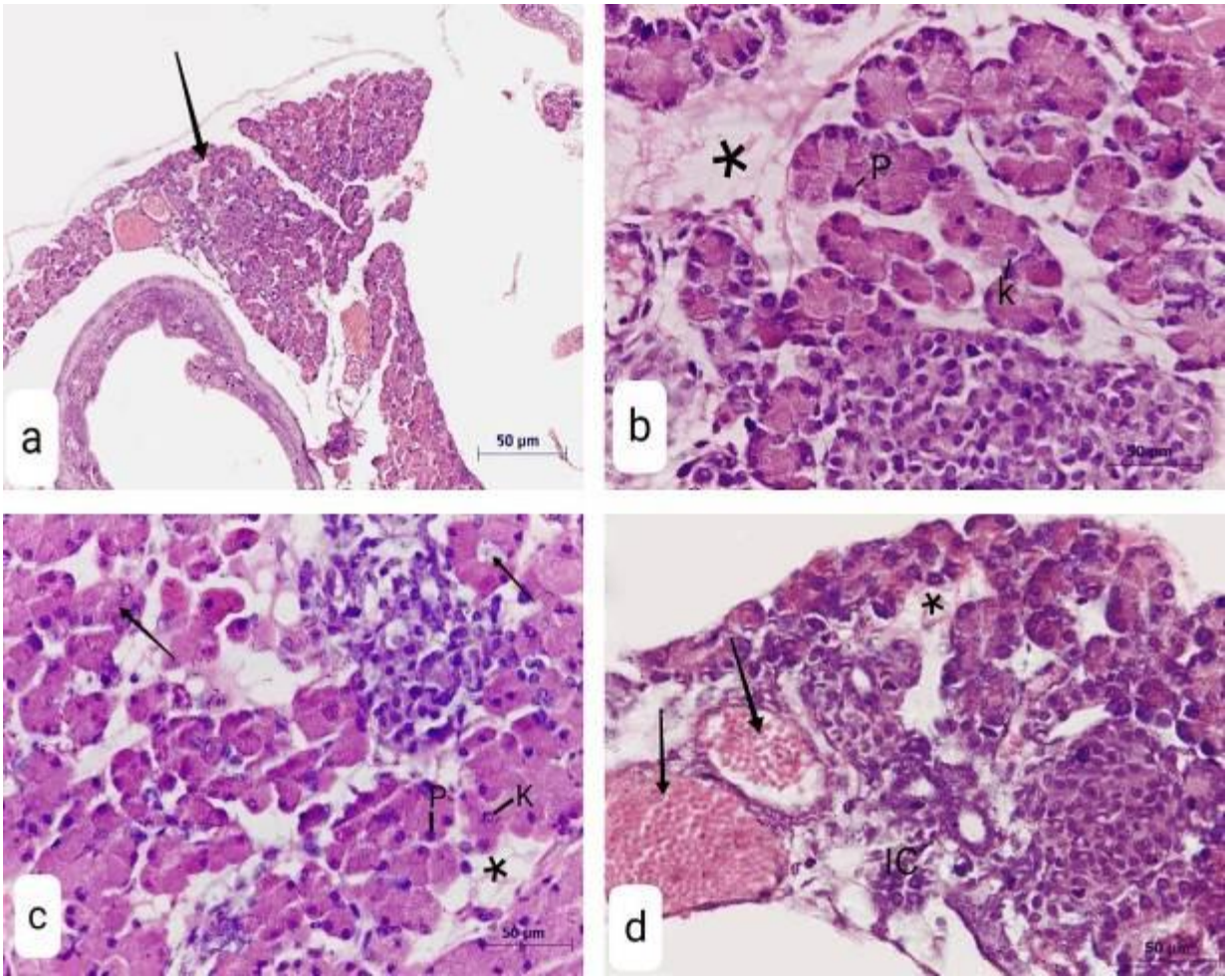

Figure (4): Photomicrographs of pancreas sections of 19-days old mice fetuses maternally treated with 3 mg/kg of DS during pre-implantation and implantation period; GDs (1-6) displaying. a- whole affected pancreas (arrow). **b & c-** vacuoler degenerations of the acini (arrows). Acinar cells with either pyknotic (P) or karyolysed (K) nuclei. Extravasations of blood from the damaged capillaries vessels forming oedema (*). **d-** congestion and damage of blood vessels (arrows) giving clear signs of haemorrhage (*). In addition, focal collection of inflammatory cells (IC) are observed.

V - Group E (3 mg/kg body weight DS maternally treated foetuses; GDs 7-14)

Maternal treatment with 3 mg/kg body weight of DS during organogenesis resulted in severe pathogenic features in the pancreas of 19-day-old mice foetuses, including acinar cell necrosis, acute inflammatory infiltration, tissue oedema, and ductal widening that caused pancreatitis and lost the normal architecture of pancreatic tissue (Fig. 5).

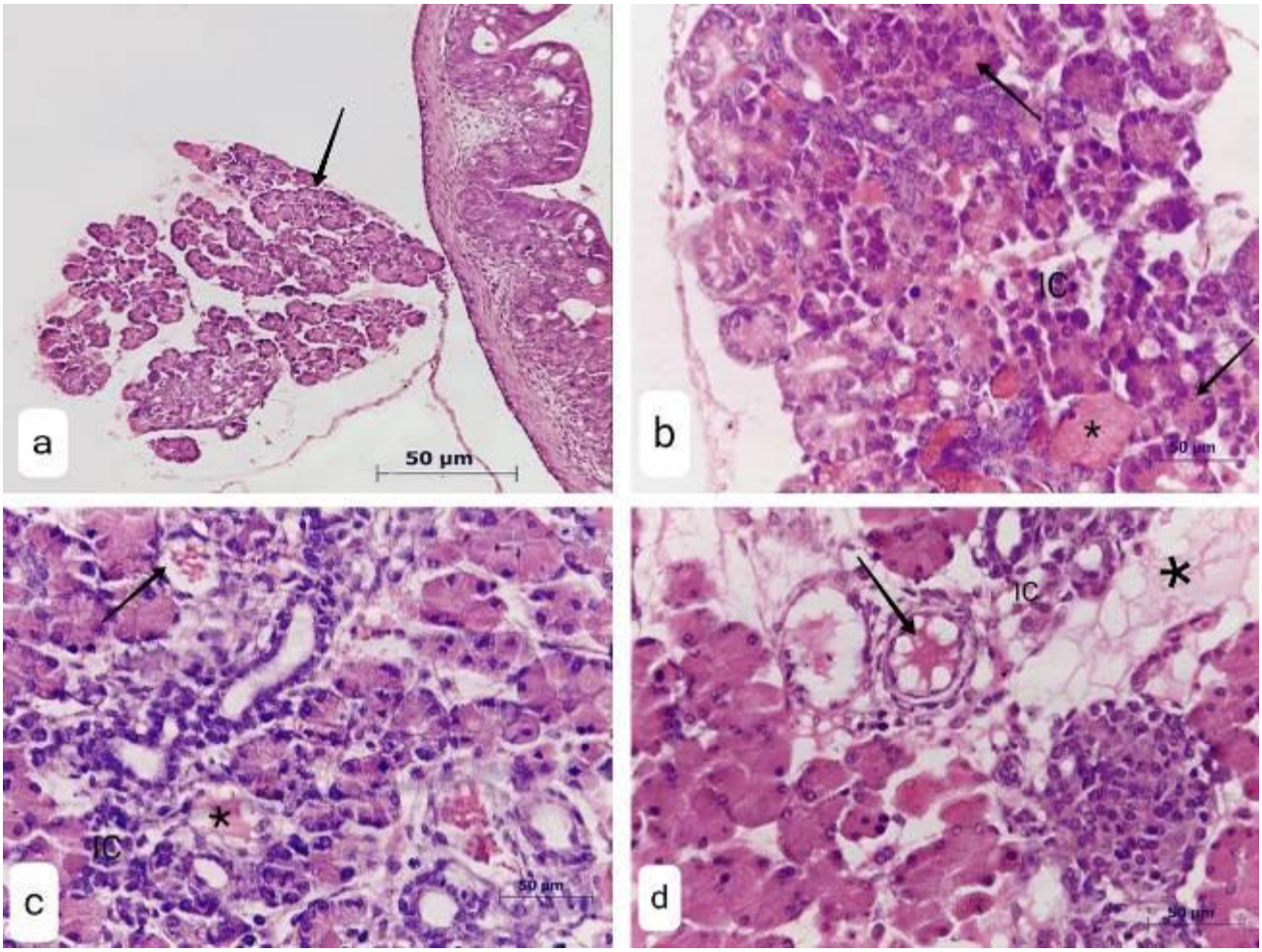

Figure (5): Photomicrographs of pancreas sections of 19-days old mice foetuses maternally treated with 3mg/kg of DS during organogenesis; GDs (7-14) demonstrating. a- severe affected pancreas (arrow), with section of stomach. **b-** severe degeneration and necrosis of the acini components (arrows). Extravasations of blood from the damaged capillaries of the blood vessels forming haemorrhagic appearance (*); in addition to inflammatory cellular infiltration (IC). **c & d** congestion and damage of blood vessels (arrow) giving clear signs of haemorrhage (*). Focal collection of inflammatory cells (IC) are also observed.

DISCUSSION

Diclofenac is often used for certain medical objectives by both pregnant and nursing women; it was stated in medical publications to have various adverse effects on body organs [(14)]**.** NSAIDs are one of the drugs to reduce pain and physical therapy [(15)]. The present investigation was constructed to study the effect of diclofenac sodium on the histological features of the pancreas of maternally treated mice foetuses. The findings of this study demonstrated unequivocally that giving pregnant mice doses of 1.5 and 3mg/kg body weight of diclofenac sodium throughout gestation (GDs 1-6) and (GDs 7-14) had considerable effects on the histological characteristics of the pancreas of maternally treated foetuses. The degenerative changes also influenced the pancreas' exocrine and endocrine systems. The obvious defects were loss of the characteristic lobular architecture, necrosis of a few acinar cells, particularly in foetuses maternally treated with double the therapeutic dosage during organogenesis, and infiltration of inflammatory cells.

The effects of several medications, particularly NSAIDs, on the pancreas and gastrointestinal tract (GIT) have been the subject of an increasing number of studies and publications over the past few decades. The majority of the effects mentioned in these research support and partially agree with the findings of current data. According to **Reyes *et al.*** [(16)], a patient who used ibuprofen every day for three weeks to manage chronic shoulder pain developed acute pancreatitis. The majority of NSAIDs have been linked to acute pancreatitis, although sulindac is the most well-known of all of them [(17)]. Following a thorough study of the literature, the authors discovered two more instances of ibuprofen-induced pancreatitis [(18, 19)]. Additionally, indomethacin was cited by **Memis *et al.*** [(20)] as the likely culprit behind acute pancreatitis in a patient who had no recognised risk factors.

Additionally, the pancreatic vasculatures of foetuses maternally treated with DS revealed obvious abnormalities, according to the results of the current investigation. These changes included obvious blood vessel dilatation and congestion, as well as haemorrhagic oedema. According to **Sabry** [(21)], who studied the effects of DS on the liver of albino mice foetuses, DS caused conspicuous alteration in the liver vasculature. In a similar trend, **Lerch *et al.*** [(22)] and **Weidenbach *et al.*** [(23)] recorded that pancreatic oedema is one of the early effects of hyperstimulation with DS and this may be due to the increased vascular permeability and hydrostatic pressure. It is important to point out that the selected doses (1.5 and 3mg/kg body weight) employed in the present study, are almost equivalent to the effective therapeutic dose and double therapeutic dose for humans. The intensity of pathogenicity was shown in mice foetuses whose mothers received a doubling dosage (3mg/kg body weight) during organogenesis, and this agrees with the observations **of Palmisano and Polhill** [(24)] that even extremely low quantities of hazardous substances can have catastrophic effects. It is a time when medications are known to have the highest risk of resulting malformations [(25)]. The central idea of the acinar cell damage hypothesis is a complex dysregulation of acinar cell activity, which leads to an unregulated release of enzymes and an improper compartmentalization of the intracellular space. These modifications may cause a sudden effusion of enzymes into adipose tissue and the interstitial space [(26)], or they may cause lysosomal hydrolases to activate intracellular enzymes [(27, 28)]. The effects of various etiologic factors may be mediated by microcirculatory alterations since these changes seem to occur mostly in the peripheral acinar cells of a lobule and because these cells are the farthest from the artery feeding a lobule.

The current histopathological results may potentially be related to developmental impairments brought on by DS. In mice foetuses maternally treated with DS during GDS 1-6 and GDs 7–14, **Sabry** [(21)] recorded growth retardation. Such growth retardation may be mirrored in the foetuses' pancreatic tissue, which looks to be functionally immature to tolerate the medication or its metabolites comparable to the adult would; potentially leading to such detrimental consequences.

In the current study, the therapy was administered during the whole pre-implantation, implantation, and organogenesis phases of gestation. It seems sense that a medication that affects the mother and can pass the placental barrier will also affect the foetus and/or new-born [(29)]. Diclofenac rapidly enters the human placenta during the first trimester, according to **Siu *et al.*** [(30)]. These may be among the primary reasons of the histological alterations found in the embryonic pancreatic tissue during the current analysis. There have been several suggested mechanisms to explain how NSAIDs work; **Zhao-Fleming *et al.*** [(31)] stated that NSAIDs are divided into groups according on how selective they are for COX-1 and COX-2. Also, non-selective NSAIDs inhibit both enzymes, and their negative effects are mostly brought on by COX-1 inhibition, which is linked to a disruption of vascular homeostasis. The blocking of prostaglandin production is thought to be the cause of the effects of NSAIDs [(32)]. NSAIDs are a possible teratogen because disruptions in prostaglandin homeostasis have been linked to the teratogenesis of prenatal abnormalities [(30)]. The NSAIDs ibuprofen and diclofenac also inhibit the proliferation of pancreatic acinar cells, according to **Bombardo *et al.*** [(5)], who hypothesised that long-term NSAID usage may impair the pancreas' capacity to regenerate, and because the foetus' pancreas is constantly growing and forming, administering diclofenac to it has harmful effects.

The forementioned findings demonstrated the detrimental impact of DS on the tissue of the developing mouse pancreas when administered during pre-implantation and implantation (GDs 1-6) as well as organogenesis (GDs 7-14) periods. Further research is required to validate these findings in humans because the medicine is widely used.

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