ISSN: 0067-2904

Histological Effect of Aspirin on the Stomach of Male Albino Swiss Mice (*Mus musculus*)

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Received: 2/12/ 2019 Accepted: 15/3/2020

ABSTRACT

The present study aimed to determine the impacts of Aspirin drug on the stomach of albino Swiss mice. The study sample included 10 male mice divided into 2 groups. The first group was orally administered with 0.1 mL of 0.75 mg/kg aspirin once daily, whereas the second group, the control, was treated with similar doses of distilled water. Following 60 days of successive treatment, a number of parameters was studied including difference in body weight and histopathological changes in the stomach as diagnosed after histological preparation. The results showed a significant decrease ($p<0.05$) in body weight average of the treated mice compared with the control group. The results also revealed the occurrence of several histopathological changes in the stomach of treated animals, including raptures in the epithelium lining, congestion in the muscular layer, and vasodilation. In addition, alterations such as hemorrhage, pyknosis in muscle cells, sloughing of the lining of the muscular layer, and external bleeding were observed. It can be concluded from these results that orally administered aspirin exerts negative effects on the stomach in mice.

Keywords: Aspirin, Albino Swiss, Mice, Stomach

التأثير النسجي للأسبرين في معدة ذكورالفئران البيض السويسرية (*Mus musculus*)

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الخلاصه

هدفت الدراسة الحالية الى تحديد تأثير عقار الأسبرين Aspirin في معدة الفئران البيض السويسرية . شملت عينة الدراسة 10 ذكور جرى تقسيمها الى مجموعتين . جرعت أفراد المجموعة الاولى من الحيوانات فموياً بـ0.1 مل من عقار الاسبرين تركيز 0.75 ملغم /كغم مرة واحدة في اليوم . أما المجموعة الثانية فقد جرعت 0.1 مل من الماء المقطر بوصفها مجموعة سيطرة . أستمرت عملية التجريع 60 يوم متتالي . تم دراسة بعض المعايير شملت التغيرات في اوزان الفئران , أذ سجلت اوزانها قبل التجربة وبعد فترة التجريع , كما تم تشخيص التغيرات النسجية في معدة الفئران المجرعة بعد اجراء التحضير النسجي لها , ومقارنة النتائج مع مجموعة حيوانات السيطرة . بينت نتائج الدراسة حصول انخفاض معنوي عند مستوى $P<0.05$ في معدل اوزان جسم الحيوانات المعاملة بعقار الاسبرين تركيز 0.75 ملغم /كغم بعد فترة التجريع , مقارنة بمعدل اوزان جسم حيوانات مجموعة السيطرة . وقد اظهرت نتائج الدراسة حصول عدة تغيرات نسجية في معدة حيوانات المعاملة شملت تمزق الطبقة الظهارية المبطنة للمعدة, احتقان وتوسع في الاوعية الدموية في الطبقة العضلية , نزف دموي مع حصول تغلظ في نوى الخلايا العضلية ، وانفصال الطبقة الظهارية عن الطبقة العضلية

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للمعدة , ووجود نزف دموي خارجي . من هذه النتائج أن التجريع الفموي للاسبرين له تأثيرا سلبيا في معدة الفئران المجرعة به .

INTRODUCTION

Aspirin (acetylsalicylic acid) is one of the non-steroidal anti-inflammatory drugs (NSAIDs) [1], with pain reducing, antipyretic and anti-inflammation characteristics [2] . Aspirin has a molecular weight of 180 Dalton and a molecular formula of C9H8O4 [3].

Aspirin is one of the most globally popular drugs that have been saving lives of billions of humans from diseases such as fever, heart attacks, strokes, rheumatism pains, ischaemia, and others. It is still considered as a favorable treatment in terms of the desired activity over its alternatives such as the anticoagulant warfarin [4].

This group of medicines has been continuously used for about 2500 years [5] as they are effective in treating many diseases, especially chronic ones such as rheumatoid arthritis and osteoporosis [6]. These uses of these drugs have been extended to involve various diseases in humans and animals, due to their broad activities as well as the differences among them in type, structure, and time of effect [7]. Hence, they have been employed in the treatment of more globally common cases [8] such as headache, cramps, backaches, tendonitis, bursitis, sprains, and pains associated with menses and injuries [9].

The use of these drugs is not free of side effects and toxicity [10], especially when used for long periods [11]. Previous studies showed impacts on the tissues and organs of the body [12], including ulcers in the intestine [13], while kidney function is also affected by the use of such drugs [14]. Other studies have warned pregnant women of the risk of miscarriage because these drugs affect embryos during the early stages of development [15]. Aspirin also causes damages to the stomach and intestinal lining leading to the development of erosions or ulcers associated with bleeding [16]. Topical and systemic effects of aspirin in the gastrointestinal mucosa are associated with mucosal damage in the upper and lower gastrointestinal tract [17]. The risk of upper gastrointestinal bleeding with aspirin is increased with old age, male sex, ulcer history and concomitant medication with NSAIDs, cyclooxygenase 2 selective inhibitors, corticosteroids or other antithrombotic agents [18]. In some patients, the cardiovascular benefits of low-dose aspirin might be overcome by the risk of gastrointestinal complications, but withdrawal of aspirin therapy can precipitate a cardiovascular event.

Despite the widespread use of aspirin, the mechanisms of its action are not fully known. It is believed that the basic mechanisms involve the inhibition of prostaglandins synthesis in the body [19]. Aspirin is a non-selective drug for COX enzyme, with its activity in reducing pain is caused by its inhibition of the enzymes COX-1 and COX-2 in the cells of the central nervous system, along with its ability to spread in the cerebrospinal fluid very quickly [20].

Aspirin is a cyclooxygenase (COX -1 andCOX -2) inhibitor during the process of the synthesis of an important physiological compound called prostanoids from arachidonic acid, where it competes with the acid for the binding with the enzyme [21].

Prostaglandins are hormone-like compounds which form naturally in the cells of the body . It is widely spread in body tissues [22]. Prostaglandins are nociceptive agents that stimulate peripheral pain receptors directly or indirectly by stimulating other factors such as bradykinin, serotonin and acetylcholines. They also regulate vascular responses, apoptosis, and inflammatory conditions [23].

The stomach is a large part of the gastrointestinal tract, particularly in mammals and birds, and is responsible for storing and transporting food to the duodenum [24]. It is consisted of cardiac, fundic, and pyloric regions while, histologically, the stomach in mammals is composed of four layers: the mucosa, submucosa, muscularis, and serosa [25]. The mucosa consists of simple columnar epithelium, and a number of gastric glands which meet with the stomach lumen through gastric pits. The layer of submucosa is composed of connective tissue, blood and lymph vessels, while the muscularis layer consists of smooth muscles. The layer of serosa, which appears as thin layer, covers the outer surface of the stomach [26].

The present study was conducted to investigate the effects of aspirin on the histological structure of the stomach of Swiss white mice.

MATERIALS AND METHODS

Animals used in the study:

This study was applied to a sample of albino Swiss mice (Mus musculus) which included 10 males, with ages ranging between 8 and10 weeks and an average weight of 25-30 grams. The mice were obtained from the Animal House of the Center for Biotechnology at the University of Nahrain, Baghdad, Iraq.

Used Drug and Experimental Design:

Uncoated aspirin, produced by the State Company for the Manufacture of Medicines and Medical Supplies in Samarra, Iraq, was obtained in the form of tablets at a concentration of 100 mg / kg per tablet. Of these tablets, a concentration of 0.75 mg / kg was used in the research experiments, which was orally administered by a special syringe to ensure that the animal receives the full dose. The treatment solution was prepared from the stock solution by dissolving 150 mg of the aspirin tablet in 100 ml of distilled water.

Males were divided into two groups (5 males per group). Members of the first group were orally dosed with 0.1 ml per 10 g of animal body weight of the 0.75 mg / kg aspirin for 60 consecutive days. The second group was considered as a control and dosed with 0.1 ml of distilled water.

Weight Measurements

The body weight of the animal was taken before and after the experiment using a normal lab balance and the difference between the two weights was recorded.

Histopathological Changes

After the end of the treatment, the treated animals were dissected and the stomach was excised. A part of the fundic region was cut, fixed with formalin 10% and transferred to ethanol 70%. After fixation, stomach sections were processed, wax blocks were produced and slides were prepared and stained with hematoxyline and eosin (H&E). The slides were examined and photographed using a compound light microscope (Meiji) with a camera [27]. The results were compared with those of the control group animals.

RESULTS AND DISCUSSION

The results in Table-1 show a significant decrease ($p<0.05$) in body weight averages of mice orally treated with the concentration of 0.75 mg/kg of Aspirin for 60 days, compared with the control group. Aspirin treatment was previously shown to reduce body weight, reverse glucose intolerance, and depress hepatic lipid accumulation in mice. The effects also included re-sensitized insulin/Akt signalling and activated AMPK signalling, with enhanced level of hepatic PPAR-α and PPAR-γ levels and activated p38 MAPK signaling. Furthermore, aspirin reduces Wnt-signalling activity via both the epigenetic regulation of Apc expression and the post-transcriptional regulation of β-catenin degradation [28]. It was demonstrated that prostaglandins, especially prostaglandin F2, have many physiological effects in the body, including metabolic effects such as regulating protein synthesis in skeletal muscle cells through the activation of ribosomes during protein synthesis [29]. This result agrees with another study [30] that showed that proteolysis in muscle cells increases due to the effect of NSAIDs, including aspirin that reduces muscle protein anabolism due to its inhibitory effect on prostaglandin F2.

Table 1-The effect Aspirin on the average body weights in mice after 60 days of dosage

Treatment Concentration(mg\kg)	**Average ± Standard error(g)**	
Primary weight before the Experiment	**Final weight after the experiment**	**the experiment**
a 24.93 ± 0.38	**a 29.10± 0.62**	**Control**
0.75	**a 26.38 ± 1.61**	**b 20.76 ±0.31**
LSD value	**a 3.011 NS**	***3.478**

*** Similar letters in the same column indicate insignificant differences (P <0.05) .**

In the first group, Swiss male mice orally administered with aspirin at a concentration of 0.75 mg / kg bw for 60 days were affected. The results obtained form examining the histological sections of the stomach showed several changes, represented by raptures in the lining of the epithelial layer, the

appearance of congestion in the muscular layer, and the occurrence of vasodilation (Figure-2), compared with control group (Figure-1) .

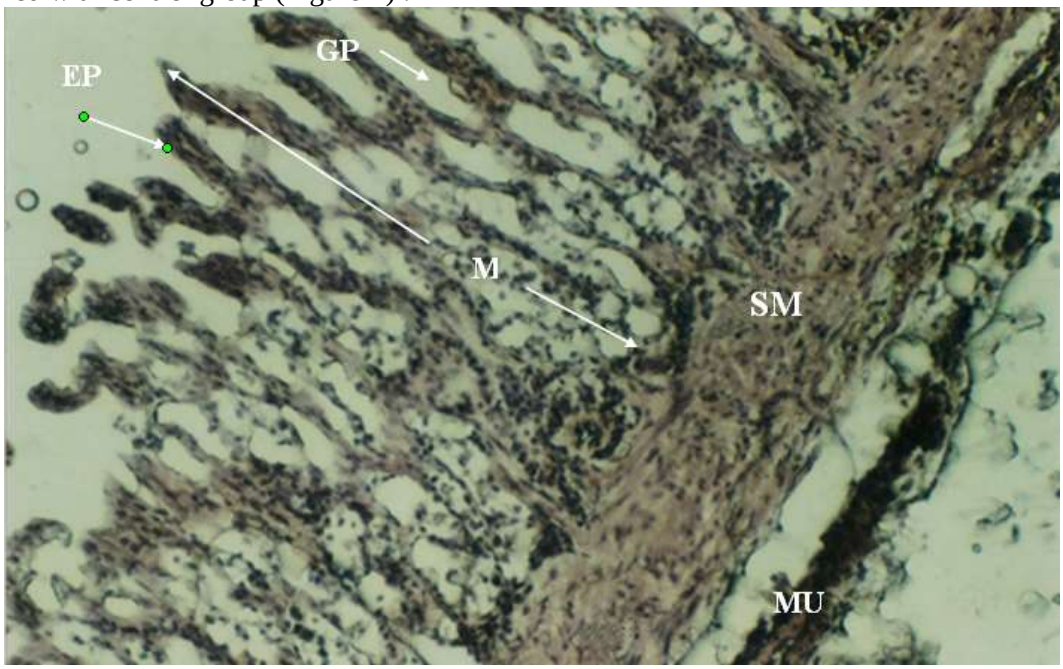

Figure 1-Cross section of fundic stomach region of the control group showing (M) mucosal layer, (EP) Epithelium, (GP) Gastric Pit (SM) , submucosal, (MU) muscle layer . (H&E stain) 200X

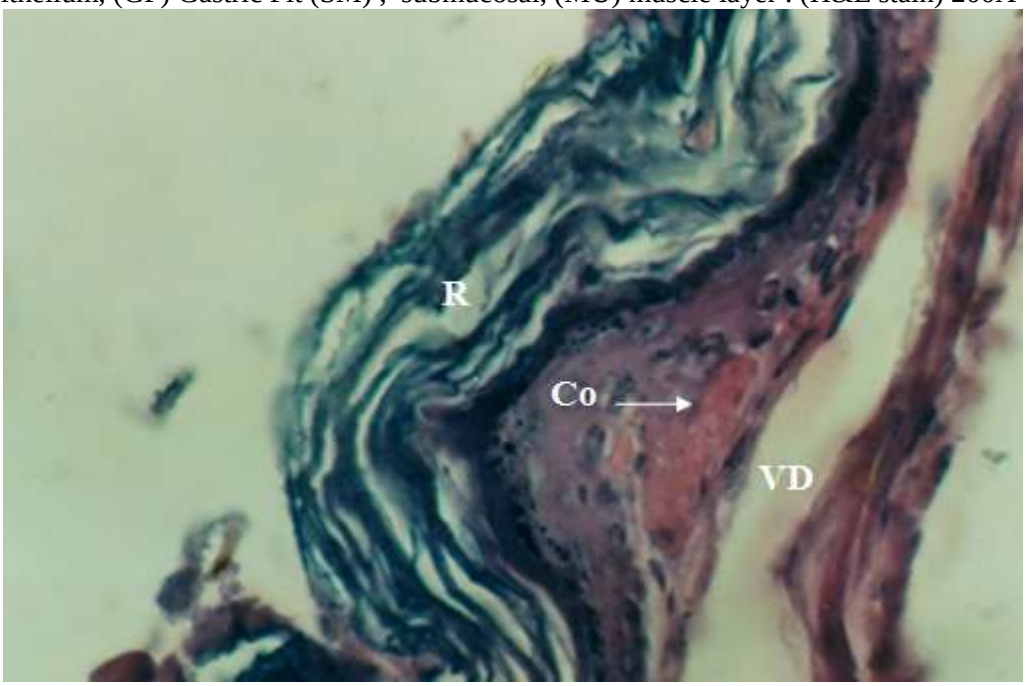

Figure 2 -Cross section fundic stomach region for Aspirin-treated animals at concentration of 0.75 mg / kg showing Congestion (Co), rupture of the lining of the stomach (R), Vasodilation (VD). . (H&E stain) 400X

These histological changes may be due to the effects of aspirin at 0.75mg / kg of body weight on stomach tissues. Many stomach lining cells were reported to be damaged and killed due to the inhibitory effects of prostaglandins on the endothelial lining of the arterioles that feed the stomach cells. Prostaglandins, especially E2, are known to stimulate arterioles to expand, whereas the observed inhibition leads to constriction of these arterioles, which reduces their supply of blood and thereby affects cell nutrition and finally leads to cell death [31].

In addition, hemorrhage, pyknotic nuclei in the muscular layer cells, as well as the sloughing of the stomach lining from the muscle layer were observed (Figure-3). The results also demonstrated the occurrence of external bleeding (Figure-4). In previous studies [32], 1 mg/kg of aspirin administered to the rat decreased the formation of thrombi in a significant way. In fact, suppression of platelet aggregation and prolongation of bleeding time in the presence of aspirin at high doses is related to the inhibition of the metabolism of arachidonic acid to thromboxane A2. This effect occurs due to the irreversible acetylation of the platelet enzyme cyclooxygenase where the inhibition of the vascular cyclooxygenase leads to the loss of the protective effect of prostacyclin [33].

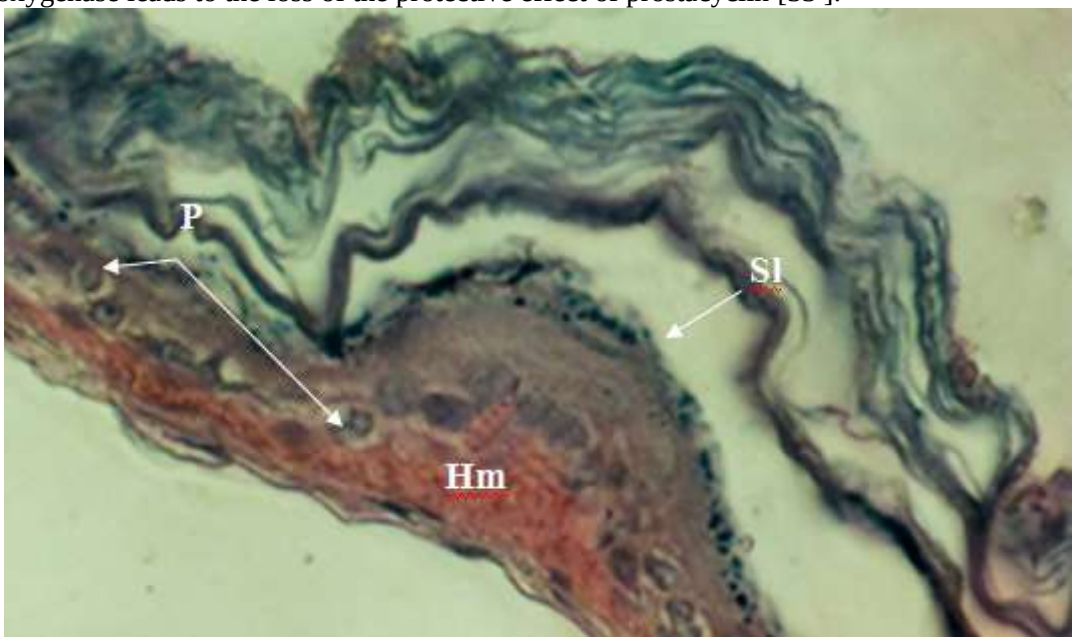

Figure 3 -Cross section of fundic stomach region of Aspirin-treated animals at 0.75 mg / Kg showing hemorrhage(Hm), Pyknosis nuclei (P) , Sloughing of gastric lining from muscle layer (Sl)). (H&E stain) 400X

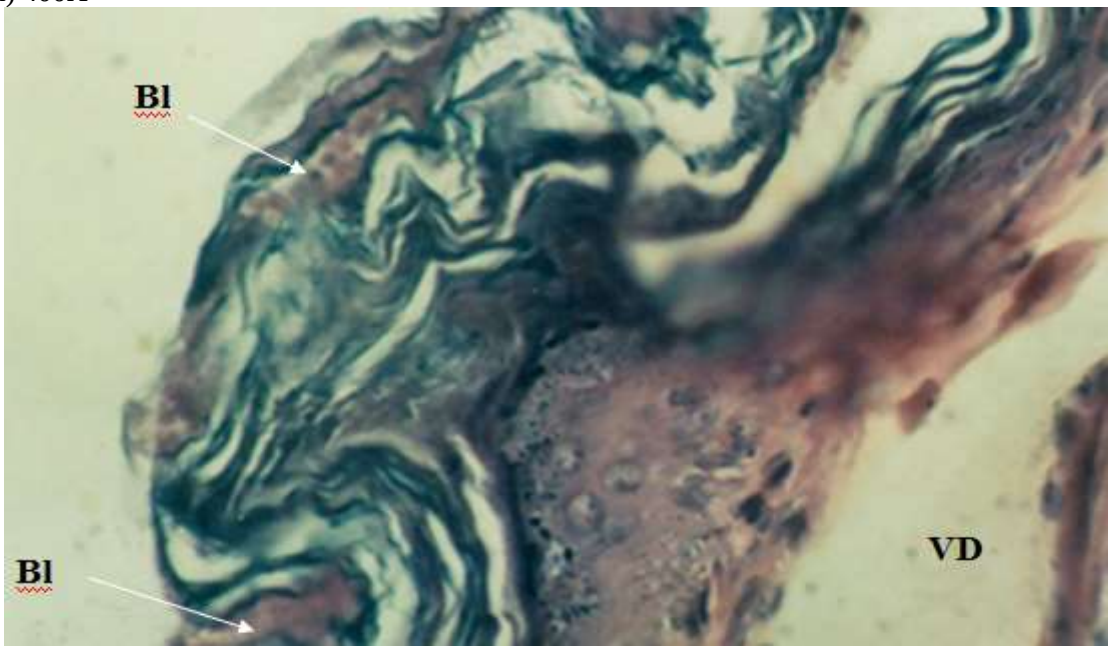

Figure 4-Cross section of fundic stomach region for Aspirin-treated animals at 0.75 mg / Kg showing Bleeding (Bl), Vasodilation (VD). . (H&E stain) 400X

The continued use of aspirin in the treatment of medical conditions at a dose of 300 mg / kg per day for 40 days was reported to increase the incidence of gastro-intestinal ulcers as a result of erosion of the epithelium lining of the digestive system [34].

The results of the present study are consistent with previous findings [35] of ulcers occurring within 30 days in people taking aspirin at a dose of 80 mg / kg per day. Another study [36] showed gastric ulcer in people aged 50-74 years when taking aspirin at a daily oral dose of 325 mg / kg for 7 consecutive days. This was attributed to the fact that aspirin inhibited the production of prostaglandin E2. In the cells of the epithelial layer lining of the stomach, prostaglandin E2 protects from the effects of the gastric acid medium represented by the hydrochloric acid HCl secreted by the cells of that layer. It also stimulates the secretion of gastric mucus that forms the protective layer, preventing the occurrence of ulcers. Previous research [37] found that the use of aspirin in high concentrations leads to bleeding due to inhibition of prostaglandins E2, E12, and thromboxane, that are responsible for ion regulation processes and blood flow in the stomach. Our results are also consistent with those of another study [38] which demonstrated the characteristics of aspirin effects on the stomach. In conclusion, our study indicates that aspirin has negative effects on the stomach of male mice through the induction of histopathological changes, which may lead to ulcers.

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