Histological Effect of Aspirin on the Stomach of Male Albino Swiss Mice

(Zainab Karim Al - Timimi)

Department of Biology, College of Sciences for Women, University of Baghdad, Baghdad, Iraq

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 ذكور الفئران البيض السويسري (Mus musculus)

زينب كريم التميمي

قسم علوم الحياة، كلية العلوم للبنات، جامعة بغداد، بغداد، العراق

الخلاصة

هدفت الدراسة الحالية إلى تحديد تأثير عقار الأسبرين Aspirin في معدة الفئران البيض السويسري. تم توزيع عينة الدراسة 10 ذكور تقسموا إلى مجموعتين. جرعت أفراد المجموعة الأولى من الحيوانات فموياً بـ 0.1 مل من عقار الأسبرين تركيز 0.75 ملغم/كغم مرة واحدة في اليوم. أما المجموعة الثانية فقد جرعت 0.1 مل من الماء المغطر بوصفها مجموعة سيطرة. أجريت عملية التجربة 60 يوم متتالي. تم دراسة بعض المعايير مثل التغيرات في وزن الفئران قبل البداية وبعد فترة التجربة، وتم تشخيص التغيرات النسجية في معدة الفئران بعد إجراء التحضير النسجي لها، ومقارنة النتائج مع مجموعة حيوانات السيطرة. في نتائج الدراسة حصول اختلاف معنوي عند مستوى 0.05 في معدل وزن حيوانات التجربة. وقد أظهرت نتائج الدراسة حصول عدة تغيرات نسجية في معدة الفئران مثل تورم الطبقة العضدية، وانقباط وانفصال الطبقة المعدنية، وانزلاق الدم في نوى الخلايا العضدية، وانفصال الطبقة المعدنية عن الطبقة العضدية.
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 prostanooids 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 and 10 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 Nahra, 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 | Average ± Standard error(g) |
|-----------|-----------------------------|
| Concentration(mg/kg) | Final weight after the experiment |
| Primary weight before the experiment | |
| a 24.93 ± 0.38 | a 29.10± 0.62 |
| 0.75 | a 26.38 ± 1.61 |
| LSD value | a 3.011 NS |
| Control | b 20.76 ±0.31 |

* 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.

REFERENCES
1. Ostensen M.E, Skomsvoll J.F, and Green GA. 2014. Non-steroidal anti-inflammatory drug. Expert. Opin. Pharm. J. Sep; 5(3): 80-571.
2. Orabi SA, Abd El-Motty EZ, El-Shamma MS, Abou-Hussein SD. and Sharara FA . 2018. The effect of Salicylic acid and Aspirin Treatments on Enzymes Activity and Fruit Quality of Clementine Mandarins Fruits during Different Cold Storage Periods. Middle East J. of Agri. 2018 Jun; 7(2): 583-593.
3. Short CR, Hsieh LC, Malbrough MS, Barker SA, Neff-Davis CA. And Davis LE. 2014. Elimination of salicylic acid in goats and cattle. Am. J. Vet. Res. 2014 Feb; 51: 1267–1270.
4. Caroline S, Julie C. and Jean-Pierre G. Acetylsalicylic acid for primary prevention of cardiovascular diseases in older patients with diabetes: do the benefits overcome the risks? . Ther. Adv. Drug Saf. J. 2015 Nov; 3(5): 213–226.
5. Scheindlin S. 2015. Historical development. Ameri . Chemi. Soci. May; 40: 202-776.
6. Warner TD. and Mitchell JA. 2011. Cyclooxygenase : new form, new inhibitors, and lessons from the clinic. The faseb. J. Jun; 18: 790-804.
7. Paterson JR, Baxter G, Dreyer J. and Halket JM . 2016. Salicylic Acid sans Aspirin in Animals and Man: Persistence in Fasting and Biosynthesis from Benzoic Acid . J. of Agri. Food Chem. 2016 Apr; 56(24): 11648–11652 .
8. Martelletti P., Farinelli I., Coloprisco G, and Pata cchioli FR. 2007. Role of NSAIDs in Acute Treatment of Headache . Drug Develop. Res . 2007 Apr; 68: 276-281.
9. Farkouh ME, Kirshner H. and Harrington RA. 2016. Comparison of lumiracoxib with naproxen and ibuprofen in the Therapeutic Arthritis Research and Gastrointestinal Event Trial. Lancet. J. 2016 Mar; 364 (9435): 84-675.
10. Ali SA. and Aljeboury GH . 2017. A Comparative Study of Amoxicillin Sensitivity Against Escherichia coli Isolates Isolated from Urinary Tract Infections . Iraqi J. of Sci. Apr; 58(1): 417-426.
11. Bennett JS., Daugherty A., Herrington D. and Green- Gncnland P. 2015. The use of Non-steroidal Anti-inflammatory Drugs (NSAIDs), A Science Advisory From the American Heart Association. J. of Am. Hea. Ass. Inc. 2015 Sep; 111: 1713-1716.
12. Farkouh ME. 2015. Review: Adverse cardiovascular effects of NSAIDs: driven by blood pressure. Sage J. 2015 Des; 2(1): 53-66.
13. Funatsu T., Chono K., Hirata T., Keto Y., Kimoto A. and Sasamata M. 2017. Mucosal acid causes gastric mucosal microcirculatory disturbance in nonsteroidal anti-inflammatory drug treated rats . Eur. J. of pharm. 2017 Jul; 554 (1, 5): 53-59.
14. Yao B., Harris RC. and Zhang MC. 2011. Interactions between 11 B hydroxysteroid dehydrogenase and COX – 2 in Kidney. Am. J. physiol. Regul. Integr. Comp. Physiol. 2011 Jan; 288 : 17-1773.
15. Fiála C., Swahn M, Stephansson O. and Danielsson K G. 2016. The effect of non-steriodal anti-inflammatory drugs on medical abortion with misoprostol at 13-22 weeks gestation. J. of Hum. Repro, Jun; 20(11): 3072-3077.
16. Schwartz HI. and Dodge WE . 2016. The impact of low-dose aspirin on endoscopic gastric and duodenal ulcer rates in users of a non-selective non-steroidal anti-inflammatory drug or a cyclo-oxygenase-2-selective inhibitor. *Aliment Pharmacol Ther.*, Aug; 15; 23(10): 98–1489.

17. Sostres C. and Lanas A. 2016. Gastrointestinal effects of aspirin. *Nat. Rev. Gastroenterol. Hepatol.*, Jun 7; 8(7): 94–385.

18. Fakhry FA. 2017. Risk of Obesity on Woman Health in Baghdad City. *Iraqi J. of Sci.*, Jun; 58(4): 2041-2050.

19. Steven B. and Abramson MD. 2018. Aspirin: Mechanism of action, major toxicities, and use in rheumatic diseases. *Proc. Natl. Acad. Sci.* 2018 May; 82(21): 7227.

20. Angela P. 2017. Aspirin: The Mechanism of Action Revisited in the Context of Pregnancy. *Front. Immunol.* Mar; 199(1–2): 93–102.

21. Small RE. 2015. Diclofenac sodium. *J. of allergy and clin. Immun.* 2015 Jan; 8: 58-545.

22. Ricciotti, E. and FitzGerald, CA. 2011. Prostaglandins and Inflammation, Arterioscler Thromb. Vasc. Biol. 2011 May; 31(5): 986–1000.

23. Francisco, JA. and Clifford, J.R. 2016. Osteoporosis and Bone Biology. *Endocrinol. J. 2016 Apr; 3(23): 55-120.*

24. Treuting PM, Dintzis SM, and Montine KS. 2018. *Comparative Anatomy and Histology A Mouse, Rat and Human Atlas.* 2nd ed. Academic press is an imprint Elsevier: Mica Haley; 194 – 201 pp.

25. Mescher AL. 2013. *Junqueira’s basic histology text and atlas.* 13th ed. McGraw Hill; 301 – 309 pp.

26. Bancroft JD. and Gamble M. 2008. *Theory of practice and histological techniques.* 6th ed. Philadelphia:Churchill Livingstone;725 p.

27. Zhou Y., Liu Z., Zhang KK., Jendrusch C., Drake M. and Hao Y. 2019. Sex-associated preventive effects of low-dose aspirin on obesity and non-alcoholic fatty liver disease in mouse offspring with over-nutrition in utero. *Lab Invest.* 2019 Feb; 99(2): 244-259.

28. Silva AM, Wang D, Komar AA, Castilho BA, Williams BR. Salicylates trigger protein synthesis inhibition in a protein kinase R-like endoplasmic reticulum kinase-dependent manner. *J Biol Chem.* 2007 Apr; 72:710164.

29. Petersen SG, Miller BF, Hansen M, Kjaer M, Holm L. Exercise and NSAIDs: effect on muscle protein synthesis in patients with knee osteoarthritis. *Med Sci Sports Exerc.* 2011 Mar; 43(3): 31-425.

30. Chan AT., Manson JE., Albert CM., Chae CU., Rexrode KM., Curhan GC. 2015. Nonsteroidal Antiinflammatory Drugs , Acetaminophen , and the Risk of Cardiovascular Events. *Circulation, AHA, Jan., 113:* 1578-1587.

31. Christian D., Omar A., Vanessa D. and Francisco X E. 2013. Paradoxical Effect of Aspirin. *Thrombosis J.*, Oct; 2: 349–360.

32. Rodriguez, LA. and Diaz SH. 2013. Risk of uncomelicated Pitic Ulcer amang User of Aspirin and Nonaspirin Nonsteroidal Anti-inflammatory Drugs. *Am. J. of Epidemiolo.* Mar; 159 (1): 23.

33. Salih LA. 2017. Histological study of the Isotretinoin drug effect on the intrauterine prenatal development in the pregnant mice. *Iraqi J. of Sci. 2017 Mar; 58(3): 1601-1608.

34. Wong VS. 2015. Benefits and Risks of Contiuing Aspirin in will Piptic ulcer Bleeding. *Annals of int. Med., Feb; 152:* 1-9.

35. Byron C., Deepak L., Lanza MD., Dongs L. and Upendra K. 2017. Low- Dose Aspirin- Induced Ulceration is Attenuated by Aspirin- phosphatidyl lcholine;Arandorized Clanical Trial. *Am. J. Gastro.* 2017 Mar; 30: 32-226.

36. Carolyn PK., Fook H., Wong S. and Lau YK. 2015. Upper Gastrointestinal Bleeding During Anti-platele Therapy. *J. of Med. Diary., Mar; 13(3): 27-30.*

37. Shajar P. and Wilcox CM. 2014. Endo-Scopic Therapy for Piptic Ulcer Bleeding. *Am. J. Gastroenterol.* 2014 Apr; 10(1): 946-983.

38. Aalykke C, Lauritsen K. 2001. Epidemiology of NSAID-related gastroduodenal mucosal injury. *Best Pract Res Clin Gastroenterol., Fep; 15:* 22-704.