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Effects of acetylsalicylic acid on histological structure of myocardial tissues in rats.

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الملخص العربي:

حمض الأسيتيل ساليسيليك له تأثير مضاد للتخثر ويستخدم على المدى الطويل بجرعات منخفضة لمنع النوبات القلبية والسكتات الدماغية وتشكيل الجلطات الدموية. الهدف من الدراسة هو معرفة ما إذا كان حمض الأسيتيل ساليسيليك يمكن أن يكون لها آثار مرضية على أنسجة القلب. تم تقسيم الجرذان إلى ثلاث مجموعات: تم إعطاء المجموعة الأولى ماء مقطر كمجموعة ضابطة، المجموعة الثانية أعطيت حمض الأسيتيل ساليسيليك بجرعة 500 ملجم / كغ مرة واحدة يوميًا بواسطة أنبوب معدي لمدة 7 أيام، بينما تلقت المجموعة الثالثة 500 ملجم / كغم من الحمض مرة واحدة يوميًا لمدة 7 أيام، ثم تم إيقاف إعطاء الحمض بعد 7 أيام من المعاملة وتركت حيوانات هذه المجموعة لمدة شهر. أجري التقطيع النسيجي للمجموعة الضابطة بعد 7 أيام وبعد شهر والمجموعة الثانية بعد 7 أيام والثالثة بعد شهر ولوحظت النتيجة. أظهر الفحص النسيجي لقطاعات القلب التحلل البؤري مع احتقان الأوعية الدموية لعضلة القلب في الجرذان المعاملة بالحمض لمدة 7 أيام. في حين أظهرت القطاعات النسيجية لعضلة القلب لجرذان المجموعة الثالثة، تسلل عدد قليل من الخلايا الالتهابية واحتقان في الأوعية الدموية. أظهرت الدراسة أنه لا ينبغي استخدام حمض الأسيتيل ساليسيليك إلا بكميات محدودة لفترات محدودة حيث تظهر علامات التسمم به حتى مع الجرعة العلاجية.

Abstract:

Acetylsalicylic acid has an antiplatelet effect and is used in long-term at low doses to prevent heart attacks, strokes and blood clot. The aim of the study was to investigate whether ASA could have pathological effects on heart tissues. Rats divided into three groups: Group 1 were given distilled water as controls, group 2 treated with ASA (500mg/Kg/d) by gastric gavage for 7 days and group 3 were received 500mg/Kg/d of ASA once daily for 7 days, then ASA was stopped for this group after 7 days and completed for a month. The histological

sectioning of the control group was performed after 7 days and one month later, the second group after 7 days, and the third group after one month, then the result was observed. Histological examination of heart sections showed focal hyalinization in association with congestion in the myocardial blood vessels in rats treated with ASA for 7 days. While, sections of group three, revealed focal few inflammatory cells infiltration associated. It was concluded that, acetylsalicylic acid should be used only in limited amounts for limited periods as its signs of toxicity appeared even with its therapeutic dose.

Key words: Histological, Heart, Acetylsalicylic acid, Rats.

Introduction:

Non-steroidal anti-inflammatory drugs (NSAIDs) are very effective in the alleviation of pain, fever and inflammation, and millions of patients worldwide have found relief in their use since the discovery of the soothing properties of willow bark more than 3,500 years ago [1]. NSAIDs use is however associated with several serious treatment side effects, with considerable associated morbidity and mortality [2]. Many of these side effects may be prevented by careful consideration of the patient's risk factors and by subsequent implementation of preventive strategies [2], [3]. Some NSAIDs, particularly those of acidic nature, can directly kill epithelial cells. NSAIDs use has also been associated with the development of hypertension and edema and with exacerbation of pre-existing heart failure [2]. These complications of NSAIDs use can be explained by NSAIDs-induced inhibition of the physiologic production of vasodilatory prostaglandin in individuals with an increased activation of the renin-angiotensin and sympathetic nervous system, as is the case in hypertension or states of effective volume depletion, such as heart failure, cirrhosis, and true volume depletion [4], [5]. In these situations NSAIDs use may induce systemic vasoconstriction by blocking the compensatory release of vasodilatory prostaglandins, causing an increase in afterload and a reduction in cardiac contractility and cardiac output [2]. Inhibition of prostaglandins results in early damage to the endothelial cells [6]. Furthermore, at least three different types of nephrotoxicity have been associated with NSAIDs administration [7], [8]. These include acute renal failure which occurs within hours of a large dose of a NSAID analgesic nephropathy which occurs from chronic consumption of NSAIDs [4]. The undesirable side effects of NSAIDs include ulcers, internal bleeding, kidney failure, and increased risk of heart attack and stroke. Some of these side effects may be due to the oxidative stress induced by NSAIDs in different tissues. NSAIDs have been shown to induce reactive oxygen species (ROS) in different cell types including cardiac and cardiovascular related cells. Increases in ROS result in increased levels of oxidized proteins which alters key intracellular signaling pathways. One of these key pathways is apoptosis which causes cell death when significantly activated [9].

Acetylsalicylic acid (ASA) is rapidly metabolized to salicylate, and within 2 or 3 h little if any ASA can be detected in the blood [10]. ASA has analgesic, anti-inflammatory, and antipyretic properties. It is used in adults for the relief of mild to moderate pain such as headache, dysmenorrhea, myalgias, and dental pain [11]. ASA is widely used for the prevention and treatment of cardiovascular disease because the antiplatelet therapy with ASA has been reported to be effective in lowering the risk of developing cardiovascular deaths with limited amounts, nonfatal myocardial infarction and stroke in patients with a history of myocardial infarction, unstable angina, non-hemorrhagic stroke or transient ischemic attack [12]. Moreover, ASA also has an antiplatelet or anti-clotting effect and is used in long-term at low doses to prevent heart attacks, strokes and blood clot formation in people at high risk for developing blood clots [13]. However, the undesirable side effects of high doses of ASA include ulcers, internal bleeding, kidney failure, and increased risk of heart attack and stroke [9].

The objective of the present study was to investigate the side effects of administration of high doses of acetylsalicylic acid induced myocardial tissues in rats.

Materials and Methods:

Experimental design:

Eighteen male albino rats were used and their average weight 200-250g. These animals were obtained from experimental animal house. All animals were maintained under standard condition in the laboratory at least ten days before use with standard food and tap water were provided ad libitum throughout experiment. They were divided into three group six animals in each group.

- **Group 1 (Control group):** Six animals received distilled water.
- **Group 2:** Six animals received 500mg/Kg of acetylsalicylic acid once daily by gastric gavage for 7 days.

- **Group 3:** Six rats received acetylsalicylic acid (500mg/Kg once daily for 7 days), then acetylsalicylic acid was stopped after a week and completed this group without ASA and taken distilled water for a month.

At the end of experiment, animals were sacrificed and hearts removed from each rat, then fixed by 10% buffered formalin. The histological sectioning of the control group was performed after 7 days and one month later, the second group after 7 days, and the third group after one month, then the result was observed. The slides were stained by Hematoxylin and Eosin [14].

Results:

Light microscopic examination of heart sections obtained from the control group stained with Hematoxylin and Eosin showed normal histological structure of the myocardium (Fig. 1).

In addition, examination of heart sections that rats treated for 7 days showed focal hyalinization in association with congestion in the myocardial blood vessels (Fig.2). On the other hand, sections of ASA-treated rats were given distilled water for one month, revealed focal few inflammatory cells infiltration associated with focal hyalinization and congestion in the blood vessels (Fig.3 and 4).

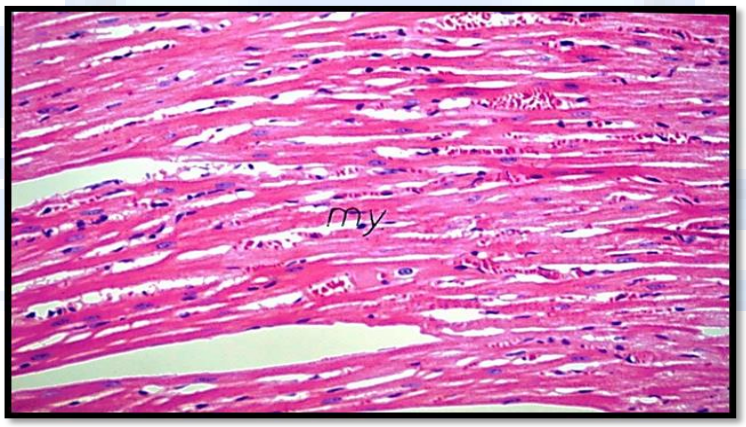

Figure (1):- Heart section of rat in normal control group showing normal histological structure of myocardial bundles (my) (H&E, 400x magnifications).

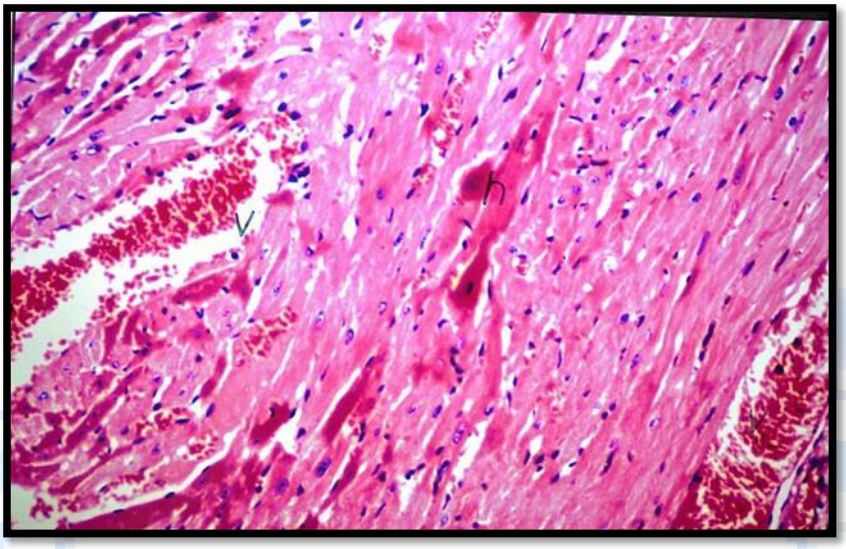

Figure (2):- Heart section of rat treated with ASA at a dose of 500 mg/kg for 7 days showing focal hyalinization in myocardium (h) with congestion in myocardial blood vessel (v) (H&E, 400x magnifications).

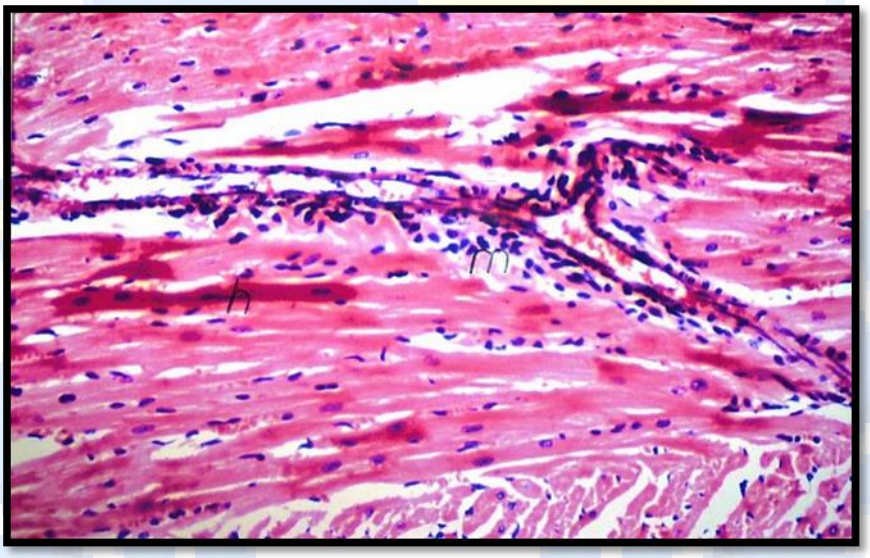

Figure (3):- Heart section of rat ASA-treated with distilled water for one month showing focal inflammatory cells infiltration (m) with hyalinization of myocardium (h) (H&E, 400x magnifications).

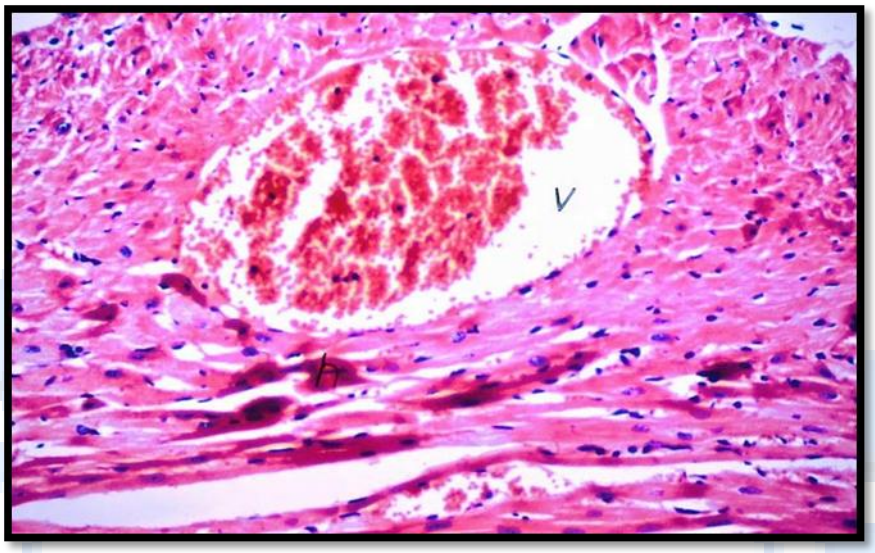

Figure (4):- Heart section of rat ASA-treated with distilled water for one month showing sever congestion of myocardial blood vessel (v) (H&E, 400x magnifications).

Discussion:

The undesirable side effects of NSAIDs include ulcers, internal bleeding, kidney failure, and increased risk of heart attack and stroke [9]. Acetylsalicylic acid irreversibly acetylates a key serine residue of cyclooxygenase (COX), blocking COX activity. As a consequence, production of prostanoids (PG, Tx) from arachidonic acid and from prostaglandin H2 (PGH2) declines, and the physiological functions that the prostanoids subsume are impaired [10]. COX activity returns to normal and the physiological actions of the prostanoid products of the COX reaction are restored only if COX is regenerated in cells or if new cells are produced. ASA irreversibly acetylates a key serine moiety of platelet cyclooxygenase-1 (COX)-1, reducing COX-1-derived synthesis of thromboxane (Tx) A2, a potent platelet aggregator and vasoconstrictor [15]. Prostaglandins are the members of a group of lipid compounds derived enzymatically from fatty acids. They are rapidly metabolized, act locally and are involved in many processes that cause inflammation after injury or illness, regulate the constriction of the uterus, affect constriction and relaxation of blood vessels, and are involved in the aggregation of blood platelets [2].

In this study the result, heart sections showed focal hyalinization in association with congestion in the myocardial blood vessels in rats treated for 7 days. While, sections of treated rats were given distilled water for one month, revealed focal few inflammatory cells infiltration associated with focal hyalinization and congestion in the blood vessels. This is confirmed with the suggestion of [9] who reported that the risk of atrial fibrillation, heart failure, myocardial infarction, and other cardiovascular conditions also increased in patients who used NSAIDs and increased the rate of cardiovascular events like cardiovascular death, and stroke. Moreover, the different trials showed for [16], [17] that several NSAIDs increased the risk of cardiovascular disease at high doses. Klaunig and Kamendulis, [18] suggested that the NSAIDs showed to associated with increased reactive oxygen species (ROS) production. ROS levels and the redox state of a cell are considered to be important in the dysfunction of various biological signaling pathways. The formation of ROS via the reduction of molecular oxygen or by the oxidation of water leads to the formation of free radicals such as superoxide anion ($O_2^{\cdot -}$), hydroxyl radical (·OH), and hydrogen peroxide (H_2O_2) [9]. Furthermore, the mitochondria-dependent overproduction of ROS has been reported under numerous pathological conditions including myocardial heart failure, inflammatory diseases, cancer, hypertension, and diabetes [19], [20]. In the heart the main producer of ROS is the mitochondria. Under normal physiological conditions, mitochondria generate ROS as a consequence of aerobic respiration. During aerobic respiration about 5 % of O_2 consumed via aerobic reaction is converted into ROS [18]. In myocardial heart failure, cardiomyocytes have been shown to be targeted by excessive ROS generation [19]. Oxidative stress arises when the oxidant production (sum of all the ROS) surpasses the antioxidant capacity in the cells [9]. Bjarnason *et al.* [21] stated that ASA and other NSAIDs cause damages through inhibition of prostaglandin synthesis and by producing microcirculatory injury.

Conclusion:

Acetylsalicylic acid, one of the widely used non-steroidal anti-inflammatory drugs, is probably the most highly consumed pharmaceutical product in the world and because they can easily be bought over the counter. So, the present study recommended that,

acetylsalicylic acid should be used only in limited amounts for limited periods as its signs of toxicity appeared even with its therapeutic dose.

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