REVIEW ARTICLE

Antimitotic Study of Acetyl Salicylic Acid (Aspirin) on Male Rats (*Rattua-Rattus*)

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ABSTRACT

The effect of oral administration of 200 mg/kg BW of aspirin on a mitotic study in albino male rats were investigated, the aspirin and colchicine drugs were administrated for two hours and the samples were collected for studies. The results showed that treatment of rats for two hours with 200 mg/kg BW of aspirin orally caused reduction in cell division at metaphase compared with treated rats with 0.5 mg/kg BW from colchicine intraperitoneal (IP), (control group).

Keywords: Antimitotic study, Aspirin, Aspirin anti- cancer, NSAIDs.

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INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, naproxen are extensively used as analgesics and anti-inflammatory agents and produce their therapeutic effects by inhibiting prostaglandin synthesis.[1] Aspirin and other NSAIDs block the formation of colon cancer in experimental animals and decrease the incidence of colorectal cancer in humans.[2] Aspirin, also known as acetylsalicylic acid, first isolated by a German chemist called Felix Hoffmann, is a salicylate drug, often used as an analgesic to relieve minor aches and pains as an antipyretic to reduce fever and as anti-inflammatory medication.[3] Aspirin is used to treat several conditions, including fever, pain, rheumatic fever and inflammatory diseases such as arthritis, pericarditis, and Kawasaki disease.[4] Aspirin has been reported to be used in preventing heart attacks and strokes.[5] Aspirin, one of the widely used drugs, is probably the most highly consumed pharmaceutical product globally. Recently, aspirin has gained greater importance as an analgesic and as a cardio-protective drug.[6] Aspirin is the only drug that combines a protective effect on the cardiovascular system and prevents some types of gastrointestinal tumors.[7] The study was performed to determine the mitotic activity of the aspirin drug.

Experimental Animals

White albino rats (*Rattus-Rattus*), weighing between 150–200gm were used for the experiment; they were housed in a standard light (12 light:12 dark cycle) controlled room temperature was set between 20–25°C with the provision of laboratory feed and water and libitum they were separated into two groups consisting of six animals per group.

Experimental Technique

Drugs used included colchicine (Ibn Hayaan pharm, syria), at 0.5 mg/kg; Aspirin (BRWN-Faridabad, Haryna, India) at 200 mg/kg; all the drugs were dissolved in distilled water before administration by dose to each animal in the group. It consisted of two groups, six animals for each group, and as follows:

- First treated group: The animals were treated with colchicine 0.5 mg/mL via intraperitoneal injection (IP) for two hrs.
- Second treated group: The animals were treated with Aspirin 0.5 mL orally for two hours.

The material and methods of mitotic study included some solutions. The following solutions were prepared according to Yaseen.[8]

1- Colchicine Solution

Colchicine solution was prepared by dissolving one colchicine tablet (1 mg of crystallized colchicine) in 10 mL distilled water. The solution was filtered and, then it was stored at 4°C for four days as a maximum.

2- Hypotonic Solution (0.075M KCl)

The solution was prepared by dissolving 1.1175 g of KCl powder in 200 mL of distilled water; the stock solution was stored at 4°C until used for cytogenetic study.

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3- The Fixative Solution

It was employed for cytogenetic studies, freshly made mixture of absolute methanol and glacial acetic acid in the ratio 3:1(v/v) for cytogenetic study.[8]

A - Direct Methods

The protocol of Allen *et al*.,[9] was done to study with some modification as follow:

1. Skin and muscle tissues were removed from both femurs immediately after sacrifice. Both epicondyle tips were removed with scissors and the marrow expelled, using a syringe with a 24-gauge needle with 5 mL of warm sterile phosphate-buffered saline (PBS) until the bone being clear, into a centrifuge tube. The suspension was mixed well to assure dissociation of the cells.
2. The cell suspension was treated with 0.1 mL colchicine at 37°C for 20 minutes.
3. The cell were centrifuged at 1500 rpm for 10 minutes, and the supernatant was discarded.
4. Five mL of 37°C hypotonic solution (warmed KCl) was added cell pellet and the suspension mixed thoroughly. The cells were incubated at 37°C in a water bath for 30 minutes.
5. The cells were centrifuged at 1500 rpm for 10 minutes, and the supernatant was discarded.
6. Freezer-chilled, freshly prepared fixative methanol: glacial acetic acid (3:1) was added dropwise, with initial mixing, to give a total volume of 5 mL. The cells were gently resuspended and then refrigerated at 4°C for one hour.
7. The cells were centrifuged at 1500 rpm for 10 minutes, and the supernatant was discarded.
8. Five ml of fresh fixative was added, cells resuspended, and centrifuged at 1500 rpm for 10 minutes. Then, two other consecutive washes with the fixative were made. One mL of the fixative was added to the cells after the last wash.

B - Slide Preparation and Staining

The cells were resuspended and then dropped from a height of about 0.5 m, using a Pasteur pipette on to wet, chilled, grease-free slides and allowed to dry at room temperature. The slides were stained with freshly prepared Giemsa stain for 2 minutes and left to dry at room temperature.[8] Microscopic examination under 100X objective lens was performed to determine the mitotic index (MI%) and blast index (BI%).

MI = [Number of chromosomal metaphase X100] / 1000

BI = [Number of blastocyes X 100] / 1000

RESULT

In a cytogenetic study, when treated rats with aspirin showed some divided cells (160) and cell numbers which not divided (840) (Table 1). But when treated, rats with colchicine showed that the number of cells divided (260), and the number of cells was not divided (740). Treatment with aspirin caused mitotic inhibiter.

Table 1: Effect of colchicine and aspirin on cell division.

Material/cell division	*Colchicine/ cell*	*Aspirin/cell*
The cell which not divided	740	840
The cell which divided	260	160
The percentage of cell not division	74%	84%
The percentage of cell division	26%	16%

MI% = [Number of metaphaseal dividing cell × 100] / Total number of 1000 cell

First group: Treated with colchicine

M_1 = 260 X 100 = 26% / 1000

Second group: Treated with Aspirin

M_1= 160 X 100 = 16% / 1000

DISCUSSION

In a cytogenic study, when treated animals with aspirin showed some divided cells and number of the cells which not

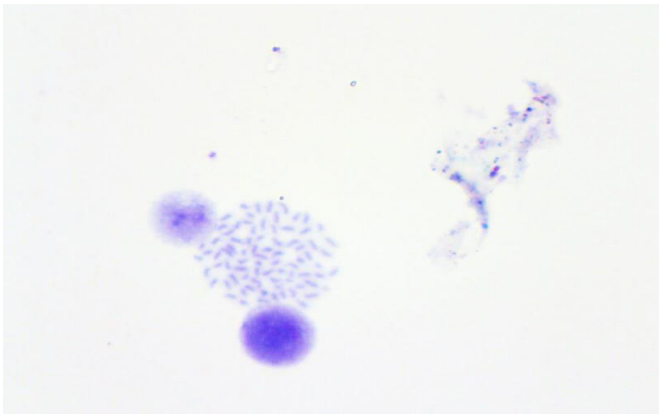

Figure 1: Chromosomal division by using colchicine (100X oil objective lens)

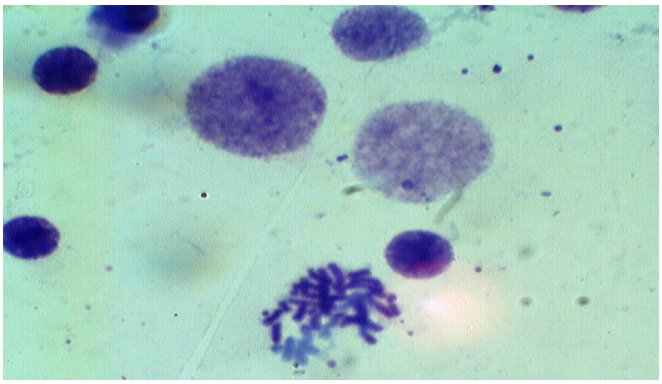

Figure 2: Chromosomal division by using Aspirin (100X oil objective lens).

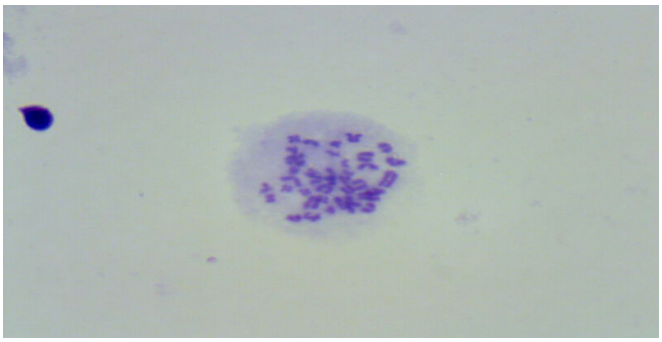

Figure 3: Normal karyotype of bone marrow cell by using Aspirin (100Xoil objective lens)

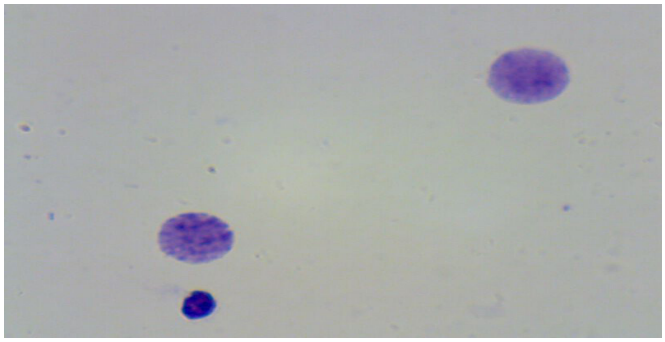

Figure 4: Normal bone marrow cell treated with Aspirin arrow Giemsa stain (100Xoil objective lens).

divided about (840) as compared with colchicine drug (740) cell, numerous epidemiological studies have demonstrated a protective effect of non-steroidal anti-inflammatory drugs, Aspirin act against several types of gastrointestinal tumors and esophageal cancer.[10]

The main effect of NSAIDs, especially aspirin, appears to inhibit cyclooxygenase enzymes (COX-I and COX-2) involved in the synthesis of prostaglandins.[11] The protective effect of NSAIDs against several gastrointestinal tumors has been well demonstrated; the long-term use of these drugs or newer and safer COX-2 selective inhibitors as chemopreventive agents has been dismissed due to their adverse cardiovascular effects.[12,13] The administration of Aspirin prevents the development of esophageal adenocarcinoma *in vivo*.[14] In one study in humans inhibition of PGE2 was detected in rectal biopsies doses (81,325 and 650 mg).[15]

The 81 mg daily from aspirin dose also suppressed PGE2 levels to the same extent as did the 650 mg, in the esophagus, it has been shown that administration of aspirin at a dose of 325 mg daily in conjugation with esomeprazole administration for 10 days resulted in lower esophageal mucosal content in patients with Barretts esophagous, where's esomeprazole alone or in combination with rofecoxib did not reduce PGE2 production.[16] In another study found no differences in esophagojejunostomy rates in rats receiving aspirin in the diet compared with a control group.[17]

Aspirin is the only drug that combines work as a protective effect on the cardiovascular system and prevents some types of GI cancer. The current study indicates that the aspirin used in this study worked as an antimitotic substance by dividing some cells in metaphase as compared with the control group (colchicine group).

DISCLOSURE STATEMENT

Conflict of interests: The authors declare that they have no conflict of interest.

Author contributions: All authors contributed equally to this work.

Ethical approval: All ethical guidelines have been followed

Sample availability: Samples of the compounds are available from the author.

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