INVESTIGATING THE EFFECTS OF ACETYSALICYLIC ACID (ASPIRIN) BY YOLK SAC INJECTION ON ROSS CHICK EMBRYO DEVELOPMENT

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ABSTRACT
Aspirin, an acetylated salicylate (acetylsalicylic acid), is classified among the non-steroidal anti-inflammatory drugs (NSAIDs). These agents reduce the signs and symptoms of inflammation and exhibit a broad range of pharmacologic activities, including analgesic, antipyretic, and anti-platelet properties. The aim of this study was to investigating effects of Acetylsalicylic Acid (Aspirin) on chick embryo development. The injection was made into the yolk with a hypodermic needle. For this aim 100 Ross eggs with 3 days old embryos selected and injected with aspirin. Drugs Aspirin did not affect the development of the embryos.

Keywords: NSAIDs, Aspirin, Chick, Embryo Development

INTRODUCTION
Non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin, cause considerable morbidity and mortality related to gastric and duodenal ulcer disease (Gabriel et al., 1991). In rheumatoid arthritis patients alone, it has been estimated that gastrointestinal (GI) toxicity related to NSAID use accounts for at least 2600 deaths in the United States annually (Pillinger et al., 1998). Thus, prevention of NSAID-induced GI damage is an important clinical issue. Misoprostol, proton pump inhibitors, and H2 receptor antagonists have been evaluated as prophylactic therapies for patients taking NSAIDs. In addition, the selective COX-2 inhibitors (coxibs) introduced a novel strategy for the prevention of NSAID-related gastroduodenal toxicity. However, the advantage of these coxibs over nonselective NSAIDs is debatable and cardiovascular toxicity has limited the use of coxibs.

Acetylsalicylic acid is the most commonly used drug in the world. It relieves headache, neuralgia, muscle and joint pain and is used in the treatment of rheumatic fever. Aspirin, an acetylated salicylate (acetylsalicylic acid), is classified among the non-steroidal anti-inflammatory drugs (NSAIDs). These agents reduce the signs and symptoms of inflammation and exhibit a broad range of pharmacologic activities, including analgesic, antipyretic, and anti-platelet properties. Aspirin was first introduced by the drug and dye firm Bayer in 1899. Aspirin and the other NSAIDs do not generally change the course of the disease process in those conditions where they are used for symptomatic relief (Gabriel et al., 1991; Pillinger et al., 1998).

Nonselective (i.e., inhibiting both cyclo-oxygenase [COX]-1 and [COX]-2) non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin, remain popular and effective drugs but can cause considerable morbidity and mortality related to gastric and duodenal ulcer disease, particularly gastrointestinal (GI) bleeding (Wallace, 1997). Even low-dose aspirin is associated with an increase in risk (McQuaid and Laine, 2006). The most common side-effect is gastric upset. Excessive use of acetylsalicylic acid can cause salicylic, a syndrome characterized by tinnitus, dizziness, blurred vision, sweating, skin eruptions, nausea, and vomiting. Intensive use of this drug can reduce the prothrombin level in the blood to the point where severe bleeding can occur.

The chick embryo is one of the most satisfactory animals on which student laboratory work may be based. It is available all over the world at all seasons of the year and can be studied with even the most modest equipment (McQuaid and Laine, 2006).
The aim of this study was to investigating effects of Acetylsalicylic Acid (Aspirin) on Ross chick embryo development.

MATERIALS AND METHODS
This experiment began 2 may 2015. 100 Ross egg were selected from Ross mother form without any kind of abnormality. For hatchery we used normal Ross chick hatch condition according to Fite et al., (1994) study. The method of this study was based on research of Fite et al., (1991). The original experimental approach (unsatisfactory) had been to explant the embryo into a moisture chamber consisting of a watch glass placed in a ring of moist cotton in a petri-dish. The medium in the watch glass would have contained the drug.

The high mortality rate of the embryos made another method advisable. The final method was quite simple. An egg of 48 hours incubation was placed in a bed of cotton in a fingerbowl. A piece of shell about one half inch square was sawed through with the point of a scalpel and removed. Every effort was made to avoid damaging the shell membrane during the process. All small particles of shell were brushed away, and the shell around the opening was wiped with a cotton swab soaked in 1% solution of iodine in 95% alcohol. The membrane was punctured with a sharp needle and cut away.

Experimental Groups
The injection was made into the yolk with a hypodermic needle. Care was taken not to injure the embryo or the vitelline (blood) vessels which cover the vitelline membrane. Each egg received 20mg of a drug dissolved in O.lcc of distilled water. The fluid was heated to 40°C before injection to avoid shocking the embryo.

A clean circular cover slip was placed over the opening in the shell. A ring of paraffin was painted on the outer edge of the coverslip to keep air from entering the egg abnormally. The egg was returned to the incubator.

At 12-hour intervals the egg was rotated about 90 degree. Care was taken that neither the embryo nor any of the extra-embryonic tissue came into contact with the observation window. The chick could be easily observed through the window for several days after the injection date. The eggs were usually opened at 10 days unless the embryo hemorrhaged or died, in which case the egg was opened at the time the condition was observed. The egg was cracked and the contents were dumped into a fingerbowl containing Locke's solution. Alter the chick embryo was observed in this solution, it was lilted into a watch glass for further study and finally was either discarded or preserved in 10% formaldehyde.

Control Groups
Three control groups were used. One group WU injected using the regular method with plain distilled water. This served as a control for the carrier. The second group received no injection. But the observation window was made in the egg. The third group allowed developing normally without a window or an injection. When an abnormal chick is compared to a control chick, the control is of the third group.

Drug Used
The drugs used were common drugs that might be found in any household drug (Aspirin) that each white tablet contains: Acetylsalicylic acid 50 gram.

RESULTS AND DISCUSSION
The results of current study presented in table 1.

<table>
<thead>
<tr>
<th>Drug used</th>
<th>Injection(N)</th>
<th>Abnormal(N)</th>
<th>Normal(N)</th>
<th>Infertile(N)</th>
<th>Dead(N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylsalicylic Acid</td>
<td>100</td>
<td>10</td>
<td>50</td>
<td>15</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 1: Results of Aspirin Injection in Embryo Development (N Mean Number)
In abnormal condition the embryo was smaller than normal group, the percentage of analysis based on above table showed the 90% were normal and 10% were smaller than control group.

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In a research conducted with Fite et al., (1994) in Oklahoma Junior Academy effects of six different drugs including aspirin were studied on chicken embryo development, they showed using aspirin or Acetylsalicylic acid 5.0gr had no side effects in embryo development and is safe drug for using (McQuaid and Laine, 2006).

In a research conducted by Mohan et al., (2012) potential toxic effects of acetylsalicylic acid upon short-term repeated oral administration in chickens were studied. In Mohan et al., (2012) study, acetylsalicylic acid at 10 mg/kg upon repeated oral administration daily for a period of five days found toxic in chickens. The most predictable and serious adverse effects associated with NSAIDs has reported in the gastrointestinal tract (Curry et al., 2005).

Gastrointestinal perforation, ulceration and bleeding have been associated with NSAID-induced depression of normal PGE2 mediated and mucosal protective mechanisms (e.g., bicarbonate and mucous secretion, epithelialisation and maintenance of mucosal blood flow) (Bertolini et al., 2001).

In other study conducted by Balong et al., (2000) effects of dietary aspirin on ascites in broilers raised in a hypobaric chamber were studied. They showed using aspirin in ascites condition had no significant differences on reducing the incidence of ascites in hypobaric chamber.

Finally we showed Aspirin did not affect the development of the embryos. May be the side effects of aspirin is only in adult cases and in embryo development had no side effects.

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REFERENCES