STUDY THE EFFECT OF AN ANALGESIC AND ANTI-INFLAMMATORY DRUG ON RAT LIVER

A THESIS

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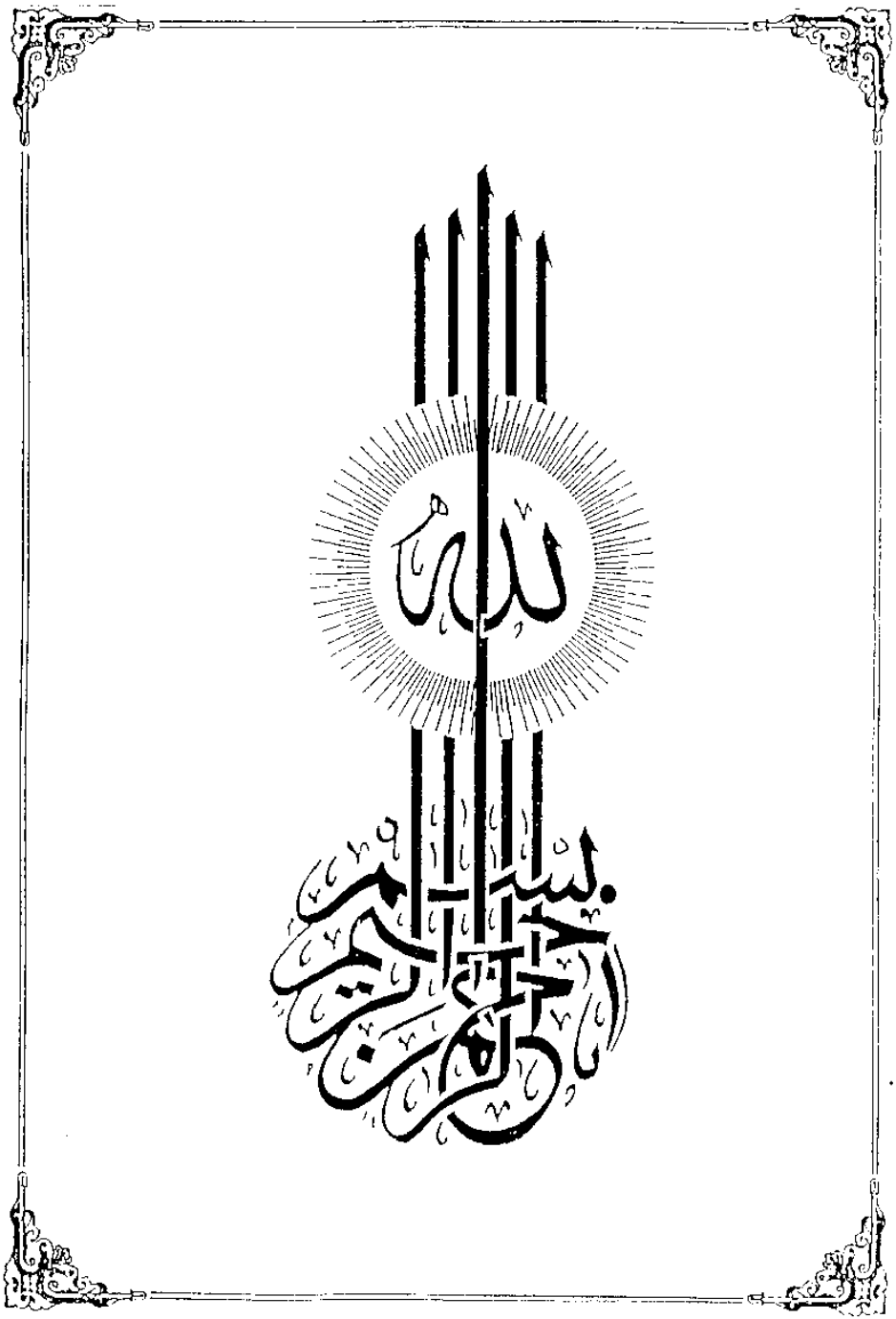

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Abstract

The hepatotoxicity of acetaminophen (A.A.) during pregnancy was investigated in both the pregnant rats and their fetuses. Different doses of A.A. were used in the present study; the therapeutic, double and triple the therapeutic doses. They were adminstered daily by gavage to the pregnant rats from the 7th till 20th day of gestation. Using the therapeutic doses of A. A., mild morphological changes as mitochondrial proliferation and dilatation were noticed in the maternal livers. In the fetal livers hypertophied Golgi complex were noticed. Moreover mild congestion and loss of glycogen were seen in the liver sections of both the mothers and fetuses.

Using double the therapeutic doses congestion was apparent with vacuolization of the hepatocytes. Apoptotic hepatocytes were detected in the liver section of both the mothers and fetuses. The nucleus was shrunken and irregular in outline with condesation of the chromatin and absent nucleolus. Some apoptotic bodies were also present either phagocytosed by the surrounding hepatocytes or in the sinusoids surrounded by macrophages. Other hepatocytes responded differently, the nucleus was intact and the organelles showed signs of degeneration. Moreover signs of cholestasis were detected.

Using triple the therapeutic doses, the maternal liver sections showed marked congestion, some hepatocytes were apoptotic, others showed degeneration of their organelles with an intact nucleus. In the fetal liver sections marked congestion was noticed with erythrophagocytosis. Some hepatocytes were seen apoptotic, while others showed cytoplasmic disintegration containing only a few organelles. The apoptotic cells were detected in all groups examined even in the controls representing apoptosis in both the hepatocytes and hematopoietic cells. Apoptosis of the latter might represent a physiological phenomena seen at birth due to the shift of the site of hematopoiesis from the liver to the bone marrow.

The level of transaminases was not significantly elevated in both the mothers and fetuses in the present study. Moreover the level of the reduced glutathione was not significantly decreased.

The gel electrophoresis of the DNA from liver specimens of the mothers receiving double and triple the therapeutic doses showed the characteristic ladder pattern of apoptosis. Fetal liver speciemens, the ladder pattern was detected in all groups including the controls. However, the intensity of the bands was proportional to the dose of A.A. administered to the pregnant rat.

Key words: Acetaminophen - hepatotoxicity - pregnancy - rat - apoptosis - transaminases - glutathione.

CONTENTS

INTRODUCTION AND AIM OF THE WORK

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Paracetamol (N - acetyl - P - aminophenol) known in the U.S.A as acetaminophen, was first described as an analgesic and antipyretic in 1893. However little interest was given to the drug till the mid 1950s when it was introduced as an analgesic antipyretic drug in the the U.S.A. Soon after, in 1960 it was included in the Britsh Pharmacopoeia (Clark, Borirakchanyavat, Davidson, Thompson, Widdop and Goulding (1973) . Nowdays it is included in 25 pharmcopoeias including Australian, Egyptian, Indian and Japanese (Reynolds, Parfitt, Parsons and Sweetman, 1993). It is classfied in the group of non steroidal anti-inflammatory drugs (NSAIDs) which have analgesic, antipyretic and weak anti-inflammatory effect.

Paracetamol had been available over the counter and had been used widely as a non prescription analgesic for the relief of common ills such as cough, headaches, achs and pain. It is as effective as asprin with fewer side effects ; it is less irritating to the gastric mucosa and does not produce acid-base changes or prolong the bleeding time (Waltman, Tricomi, Tevakoli, 1976 and Pearson, 1978). Recently Bradley (1991) stated that paracetamol in doses of 4 g. daily was as effective in the short term management of osteoarthritis of the knee as ibuprofen in doses of 1.2 g and 2.4 g daily.

The hepatotoxicity of paracetamol overdose is well known, it was first reported in rats by Boyd and Bereczky (1966). A report of such hepatic necrosis in man soon followed (Davidson and Eastham, 1966). Many case reports and animal researches were then published. The study of paracetamol hepatotoxicity and factors affecting it was, and continues to be a fruitful pursuit (Vale and Proudfoot ,1995)

Moreover some cases were reported after the chronic use of therapeutic doses (Barker, De Carle and Anuras, 1977; Bonkowsky, Mudge and Mc Murtry, 1978 and Olsson, 1978) and this was specially pronounced with the presence of underlying liver disease or alcohol abuse.

Pregnancy is frequently complicated by many musculo-skeletal disorders, such as carpal tunnel syndrome and low back pain thus the use of analgesics and anti-inflammatory drugs must be considered. Also, the peak prevalence of rheumatoid arthritis occurs in women aged 20 to 40 years. Thus anti-rheumatic medications, specially NSAIDs, are frequently used in women of child bearing age. Drug exposure may range from intermittent use of analgesic NSAIDs to constant medication with a variety of anti-rheumatic drugs.

Usually paracetamol is recommended during pregnancy over other analgesic antipyretic agents because it is safe in therapeutic dose (Berkowitz, Coutan and Mochizuki, 1981 and Hill and Kleinberg, 1984). Nowdays, paracetamol is widely used by pregnant women; Heinonen, Slone and Shapiro (1977) conducted two cohort studies on 50 and 282 mother child pairs and found that 31.6% of gravidas had taken analgesics during early pregnacy and 67.3% had used analgesics by the end of pregnancy. Also McElhatton, Sullivan, Volans and Fitzpatrick (1990) stated that an enquiry done by Teratology Information Service in the United Kingdom showed that analgesics were the group of drugs most commonly involved in both the overdose and therapeutic dose, with paracetamol alone or in combination accounting for over 60% of the analgesic drug enquiries.

Ideally, the pregnant woman should receive no drug but once the decision is made to use a drug during pregnancy, the physician should ask two questions. Firstly, has the drug been implicated in human teratogenesis and secondly, how can any potential alteration of physiology and functional deficit (Needs and Brooks, 1985).

In common with most other drugs, the technical and ethical restraints placed on investigations during the course of pregnancy has resulted in only very limited information regarding the effects of NSAIDs during pregnancy. The study of the safety of paracetamol during pregnancy was only done using epidemiological methods (case control and cohort studies) which mainly concerned the teratogenecity of the drug. However, the associated hepatotoxic effects of the drug in the mother and/or the fetus were not investigated. Moreover sparse animal data regarding paracetamol safety during pregnancy (Lubewy and Garett, 1977 and Popp and Owen, 1979) were published, none of them concerned the hepatotoxicity of the drug.

Reviewing the literature for the use of paracetamol overdose during pregnancy, only suicidal attempts using a single very large dose of the drug were recorded. Thus it has been the aim of the present work to investigate the hepatotoxicty to the pregnant rats after chronic drug intake during pregnancy using both therapeutic and overdose .

Paracetamol is known to cross the placenta (Levy Garrettson and Soda 1975, Lederman fysh, Tredger and Gamsu 1983 and Robert, Robinson, Mughal, Ratchiffe and Prescott 1984). However how much of the overdose cross the placenta and how the fetal liver deals with this insult is unkown. Thus it has been the aim of the present work to investigate the effect of paracetamol (acetaminophen) given to the pregnant rat on their fetal livers. These investigations will be done using different morphological and biochemical techniques.

Moreover, Ray, Sorge, Raucy and Corcoran (1990) had found early loss of large genomic DNA accompanied by the appearance of periodic DNA fragments during acetaminophen induced liver inury in mice. Such results had been also confirmed in cultured mouse hepatocytes by Shen, Kamendulis, Ray and Corcoran (1991). Thus it has been the aim of our work to find out such results in the rat after giving different doses of acetaminophen using DNA gel electrophoresis and in situ hybridization.