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Morphological Evidence of Apoptosis in Hepatocytes of Rats (*Rattus norvegicus*) Exposed to Arabian Incense

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Hepatocytes of rat exposed to Arabian incense revealed morphological evidence of apoptosis in their nuclei. Irregularity of the nuclear membrane was probably the initial morphological alteration. Evident chromatin condensation was the following nuclear change. Nuclear fragmentation was the subsequent nuclear apoptotic change. Each nuclear fragment was delimited with intact membrane. No evidence of chromatin lysis was noticed in the altered nuclei. Markedly shrunken nuclei with condensed chromatin were also discerned. Cytoplasmic organelles of hepatocytes with apoptotic nuclei revealed no sign of deterioration. Kupffer cells in the vicinity of the apoptotic nuclei were hypertrophied and proliferated.

Key words: Arabian incense, apoptosis, hepatocytes, ultrastructure

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INTRODUCTION

Cell death is believed to proceed through two ways, namely apoptosis and necrosis[1-4]. Apoptosis is a programmed and highly regulated intrinsic pathway which involves a cascade of molecular and biochemical events leading to cell death[4-6]. It serves as a physiological process to eliminate excessive or unwanted cells during remodeling of embryonic tissues or metamorphosis and during organ involution[7-10]. In other words, it is essential for development and tissue homeostasis[11]. This process may also play the same role in some pathological conditions, such as preneoplasia, to remove the damaged tissue cells[12-14]. The morphology of apoptosis seems to be identical regardless the cell type undergoing the apoptotic process[13]. Hepatic apoptosis was the aim of some previous studies[15-21].

Arabian incense is an oleoresin that oozes from incision in the trunks and leaves of the genus Boswellia (*B. carterri* and *B. papyrifera*) native of Arabia, Africa and India[22]. According to the local traditions, individuals may get in an intimate daily contact to the Arabian incense by inhalation of the resultant smoke. The effect of this indoor daily custom on the pulmonary tissue was the aim of our previous studies[23-25].

To the best of the author's knowledge, there is no published study focusing on the relation between exposure to Arabian incense and the occurrence of apoptosis in the liver tissue. Therefore, the present study was designed to provide a morphological evidence that exposure to Arabian incense can induce hepatic apoptosis.

MATERIALS AND METHODS

Experimental animals: Wister albino male rats, *Rattus norvegicus*, weighing 95±10 g and of the same age were used. Animals were obtained from King Saud University colony. Rats were maintained under standard laboratory conditions including diet and temperature (25°C). Water and feed were available *ad libitum*.

Experimental design: Animals were divided randomly into two experimental groups (treated and untreated control) of 16 rats each. Treated rats were exposed to 420 g of Arabian incense for 14 weeks, at the rate of 4 g/day in an exposure chamber. Untreated animals were unexposed and served as control. At the end of the experimentation, all experimental rats were anesthetized, dissected and livers were removed.

Electron microscopy: Immediately after removal of livers, tissues were diced into proper sized pieces (1 mm^3) and fixed by immersion in buffered 3% glutaraldehyde (cacodylate buffer, pH 7.2) for at least 4 h at 4°C. Tissue specimens were then post-fixed in 1% osmium tetroxide (Oso_4), in cacodylate buffer pH 7.2, for 2 h at 4°C. Dehydration of the fixed tissues was performed using ascending grades of ethanol and then tissues were transferred to epoxy resin via propylene oxide. Semi-thin sections (1 μm in thickness) were prepared for the purpose of tissue orientation and stained with toluidine blue. Accordingly, thin sections (70-80 nm) were cut on an ultramicrotome (Leica, UCT) and double stained with uranyl acetate and lead citrate. Stained tissue sections were observed with a transmission electron microscope (JEOL, 100 CX) operating at 80 kV.

RESULTS

Control animals: The hepatocytes of unexposed animals showed rounded nuclei which had regular nuclear membrane (Fig. 1). The evenly distributed fine granular chromatin (euchromatin) was the major chromatin component while the dense component (heterochromatin) was the minor and seen as clumps within the nucleoplasm as well as on the nuclear membrane. The main cytoplasmic organelles, Rough Endoplasmic Reticulum (RER) and mitochondria were regularly distributed and revealed no morphological abnormalities. Kupffer cells were of normal state with no signs of hypertrophy or proliferation.

Exposed animals: The nuclei of Hepatocytes revealed various abnormal morphological forms reflecting the successive stages of apoptosis. Probably, the initial change indicating the nuclear apoptosis was the irregularity of the nuclear membrane (Fig. 2). However, this was not accompanied with chromatin condensation and heterochromatin was still seen as little clumps on the nuclear membrane. The nucleoli of these nuclei were marginally dislocated. The subsequent nuclear change was the invagination of the nuclear membrane to form nuclear indentations of various depth (Fig. 3). The nuclear indentations were occasionally deep enough to give the impression that a part of the nucleus will separate (Fig. 4). The nuclear indentation was accompanied with condensation of chromatin with tendency of heterochromatin for more clumping and extension into the interior of the nuclei.

Fragmentation of the hepatocytes nuclei was probably the following stage of nuclear abnormalities. The apoptotic nuclei were fragmented into varied-sized pieces. However, each nuclear fragment was still delimited by intact nuclear envelope (Fig. 5).

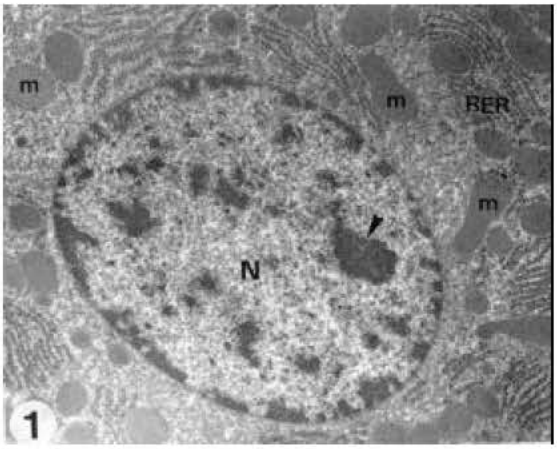

Fig. 1: Transmission electron micrograph showing a hepatocyte of unexposed animal. The Nucleus (N) has a regular nuclear membrane and evenly distributed euchromatin and occasional clumped heterochromatin. Note the prominent nucleolus (arrowhead). The Mitochondria (m) and Rough Endoplasmic Reticulum (RER) are normally distributed and reveal no morphological alterations. X 6700

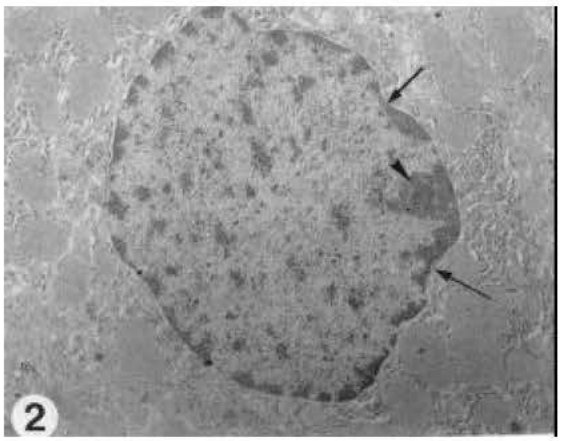

Fig. 2: Hepatocyte of an exposed animal showing obvious irregularitory of its nuclear membrane which reveals indentations (arrows). Note the margination of the nucleolus (arrowhead). Transmission electron micrograph. X 8000

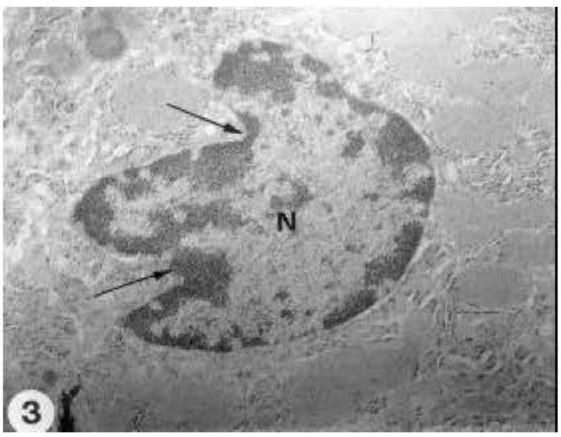

Fig. 3: Hepatocyte Nucleus (N) of an exposed animal showing deep indentations (arrow) of the nuclear membrane. The heterochromatin is more clumped on the nuclear membrane. X 14000

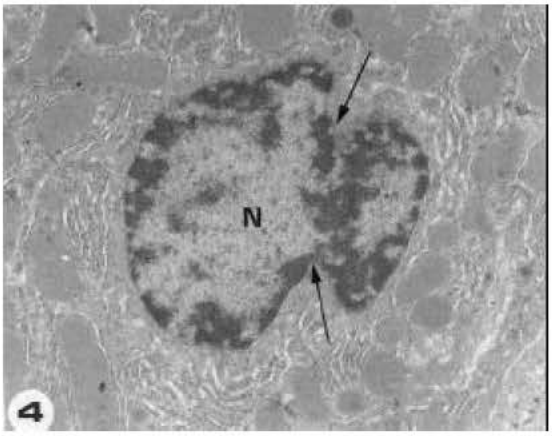

Fig. 4: More deep indentations (arrows) manifested by the Nucleus (N) of a hepatocyte of an exposed animal. The nuclear membrane indentations are deep enough to give the impression that the nucleus will be fragmented. X 10000

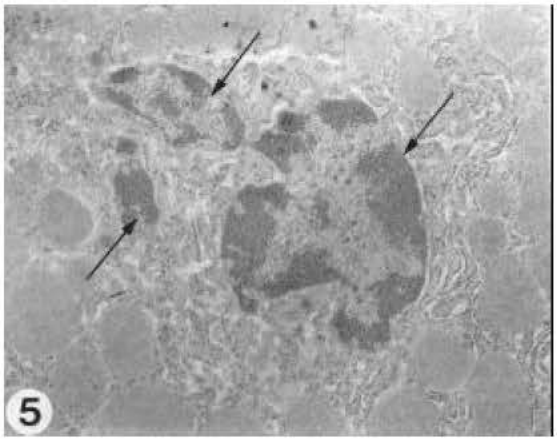

Fig. 5: Nuclear fragmentation in a hepatocyte of an exposed animal. Note the varied sized nuclear fragments (arrows). The smaller fragment has a highly clumped chromatin and larger fragment shows deep invaginations. Note that the fragments are membrane-bound with no damage of the delimiting nuclear membrane and no evidence of chromatolysis. X 14000

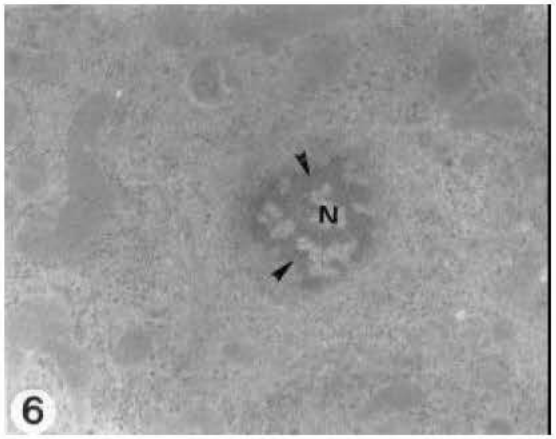

Fig. 6: Markedly shrunken Nucleus (N) of a hepatocyte from an exposed animal. The shrunken nucleus has a reticular-pattern chromatin and shows membrane indentations (arrowhead). X 14000

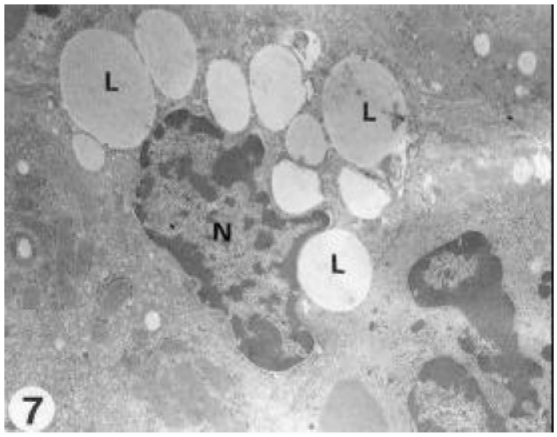

Fig. 7: Kupffer cell from an exposed animal showing accumulation of large-sized lipid droplets (L) in its cytoplasm. The Nucleus (N) of the Kupffer cell is deformed owing to pressure exerted by the lipid droplets. X 6700

Larger nuclear fragments showed more indentations of their delimiting envelope probably as an indication for further fragmentation. Chromatin condensation was more pronounced in the smaller nuclear fragments which disclosed a reticular pattern of the clumped chromatin intermingled with euchromatin (Fig. 6). Even in the evidently fragmented nuclei, no evidence of chromatin lysis (chromatolysis) was recognized. Markedly shrunken nuclei with evidently clumped chromatin were also observed (Fig. 7).

In general, hepatocytes having apoptotic nuclei showed no noticeable swelling or deterioration of their organelles including RER and mitochondria which had intact limiting membranes. Kupffer cells were activated as evidenced by hypertrophy of their nuclei and also by the existence of much debris contained in their cytoplasm. Kupffer cells impacted with large-sized lipid droplets, which deformed the nuclei, were also noticed (Fig. 7). Kupffer cells were also relatively increased in number compared with that observed in control liver tissues.

DISCUSSION

Presently as revealed by electron microscopy, hepatic cells undergoing apoptosis manifested a tendency of the nuclear chromatin for margination and condensation into dense masses which lied on the nuclear membrane. Also, the cytoplasmic cell masses were gradually shrunken. The relevant advanced nuclear changes were recognized in the form of chromatin fragmentation. These nuclear events are in common with the morphological criteria considered characteristic for apoptosis[7,14,21,26,27]. Condensation of chromatin appears to be the initial step in the present cases which showed apoptotic nuclei. In this respect chromatin condensation in apoptosis is believed to be the initiating event[28,29]. Also, nuclear condensation is considered as the most striking morphological feature of apoptosis[14,21].

Chromatin cleavage or fragmentation is supposed to be the subsequent step and this cleavage was reported to be induced by the non-lysosomal endonuclease in hepatic cell nuclei[30,31].

Different nuclei in the presently investigated hepatocytes manifested varying apoptotic events, such as irregularities of the nuclear membrane, chromatin condensation and aggregation on the nuclear membrane and fragmentation. This finding supports the conclusion that apoptotic events do not progress in a synchronizing manner[32].

Concerning the nature of the apoptotic process encountered in the present study, it is possibly typified as an induced one. Although apoptosis is a naturally occurring process, it can also be induced by external stimuli[10].

The presently described process of apoptosis was possibly employed to remove the damaged hepatocytes. Apoptosis was found to be more advantageous than necrosis for removing injured hepatocytes[2]. Necrosis results in the release of inflammatory mediators which initiate a sequence of damaging effects in the tissue. Cellular changes such as loss of plasma membrane integrity (hall mark of necrosis), swelling of cytoplasmic organelles and bursting of cytoplasmic and nuclear materials which are typical of necrotic cell death[13] were not detected in the present cases which manifested apoptotic cell death. Since the hepatic cell apoptosis occurs rapidly[33], it has been concluded that it shortens the time course of hepatic injury and its lack can lead to persistent chronic inflammatory reaction which ends by fibrosis[34].

The observed proliferated and hypertrophic Kupffer cells were most likely a response to the existence of apoptotic hepatocytes. Kupffer cells which are professional phagocytes contribute significantly in eliminating apoptotic hepatocytes[2]. Also, release of cytokines in the liver tissue after toxic injury activates Kupffer cells[35]. Thereafter, proliferated Kupffer cells may initiate the apoptotic events through their cytotoxic mediators[36].

Distribution of the presently described form of cellular apoptosis followed the pattern of solitary cells. This agrees with the description of apoptosis as a form of single cell necrosis unlike the necrotizing process which usually involves groups of cells[37]. The mechanism of apoptosis in the presently investigated hepatic tissue is possibly related to damage of DNA as do some toxins

which lead to apoptotic cell death[38,39]. However, the exact mechanism triggered the hepatocyte apoptosis in the present study is yet to be identified.

In the current study a morphological evidence of hepatocyte apoptosis induced by the exposure to Arabian incense is presented. Further studies on this subject will focus on the relevant pathogenic mechanism(s) as well as the cellular changes at the molecular level.

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