

Effect of insulin on Ultrastructural changes in liver after treating with arsenic in diabetic rats

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Abstract: Arsenic is found naturally but various anthropogenic activities have led to the increase in pollution of arsenic. Arsenic pollution is found in many countries viz. India, Bangladesh, Chile, Argentina and many more. It affects various organs of an individual like liver, kidney, skin, pancreas etc. Prevalence of diseases like diabetes mellitus has increased nowadays due to the changing lifestyle of the population. The study was carried out to find out the interaction of diabetes mellitus and arsenic in the liver of rats. It tries to find out effects on the ultrastructure in liver of arsenic treated diabetic rats.

Keywords: Arsenic, diabetes, insulin, ultrastructure, population, liver.

Introduction-

Arsenic is a suspected carcinogen for humans and animals [1]. Countries such as Bangladesh, India, [2] China, [3], Chile [4] and Argentina [5] etc. have reported a high contamination of arsenic in the ground water. Exposure to arsenic for long duration causes various adverse effects on health viz. cardiovascular disease [6, 7], liver diseases [8], skin lesions [9] also, diabetes mellitus [10]. IARC established liver as the potential organ for cancer due to arsenic toxicity [11]. Studies shows that arsenic exposure effects DNA methylation. [12]. For arsenic metabolism main site is liver so various liver disease occur in exposed individuals/humans. [13, 14, 8]. Arsenic-causes hepatic fibrosis, cirrhosis, and hepatomegaly in liver cells [8, 15, 16]. Arsenic plays a role in thioredoxin (Trx) and the apoptotic signalling pathways [17]. Reactive oxygen species (ROS) are generated by arsenic during its metabolism by activation of NADH oxidase [18]. Arsenic exposure interferes with the glucose tolerance as it disturbs the function of beta cells present in islet of Langerhans causing hindrance in insulin secretion and does not increase peripheral insulin resistance [19]. Diabetes mellitus is also correlated to arsenic exposure which has been observed in regions of Taiwan and Bangladesh [20, 21, 22]. So there seems to be association between diabetes mellitus and arsenic [10,20]. Ultrastructure changes caused by arsenic in liver of diabetic rats. Further effect of insulin on the rats have also been studied in this paper.

Material and methods

(a) Selection and maintenance of test species-

Wistar rats (male) with body weight ranging from 150 ± 20 g were obtained from Jamia Hamdard, New Delhi. These rats were kept for acclimatisation in laboratory conditions for 2 weeks (R.H. $50 \pm 10\%$ and temp $25 \pm 5^\circ$ C). These rats were kept separately in polypropylene cages and fed pelleted food obtained (Golden Feeds, New Delhi) and tap water was given to them *ad libitum* during present study.

(b) Experimental procedure and treatments

Thirty male rats were separated into six groups on a random basis (a, b, c, d, e and f) each having five rats and housed in polypropylene cages separately. An intra-peritoneal injection of alloxan monohydrate (12.5 mg per 100g body weight) was given in four groups c, d, e, f to introduce diabetes [23] a kit was used to examine blood sugar level with the help of Folin and Wu by method (Span Diagnostics, Surat, India). For administering arsenic trioxide 3 groups of rats namely b, d and f were chosen and it was administered at 4mg per 100g alternatively for 30 days. [23]. Group e and f were given insulin (0.1ml per 100 g body weight) on alternate day. Group of a rats were given normal saline to treat as controls. Arsenic trioxide was purchased from C.D.H, Mumbai Alloxan monohydrate from Loba Chemie Mumbai, and bovine insulin from U.V.S. Limited, Mumbai.

Preparation of samples –

Post 30 days exposure, experimental animals were kept starving whole night and sacrificed with the help of light ether anaesthesia, in the morning. Samples of liver were taken carefully and weighed on electronic balance. The samples were prepared for further examination by transmission electron microscopy.

T.E.M. Studies

Liver was sliced into sections of 1 cu mm and then immersed in 2.5% glutaraldehyde after that fixed in 1.0% osmium tetroxide. Post fixation the sample was dehydrated using different grade ethanol and then dipped in EPON 812 later putting sample in different changes of propylene oxide. A Philips CM 10 TEM (Transmission electron microscope) was used for Examination of the above tissue sections prepared after staining with uranyl and lead acetate at AIIMS, New Delhi (microscope facility).

Results

Liver tissue of rats used as control group was examined with TEM exhibited chromatin well placed inside the nucleus, intact ER, round shaped mitochondria and ribosomes. In liver parenchyma many glycogen bodies were observed (figure a). After treating with arsenic the various changes were found in hepatocytes as increased number of mitochondria, presence of many vacuoles (in the cytoplasm), changed shape and size of nuclei, condensed endoplasmic reticulum (figure b). TEM study of liver of diabetic rats revealed presence of vacuole and swollen nuclei (figure c). Examination of arsenic treated diabetic rats showed variable sizes and shape of mitochondria and numerous cytoplasmic vacuoles (figure d). The morphology of nucleus and endoplasmic reticulum was improved in case of insulin treatment administered to rats with diabetes mellitus (figure e). In the group where arsenic was given in the insulin treated diabetic rats there was proliferation of ER (figure f).

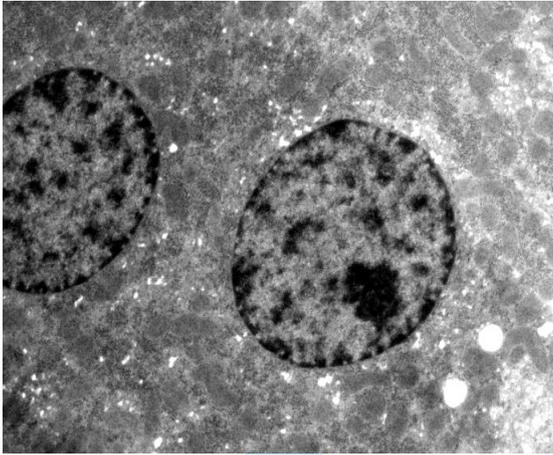


Figure a: T.E.M micrograph of rat liver (control) showing nuclei with placed nucleus (x1800)

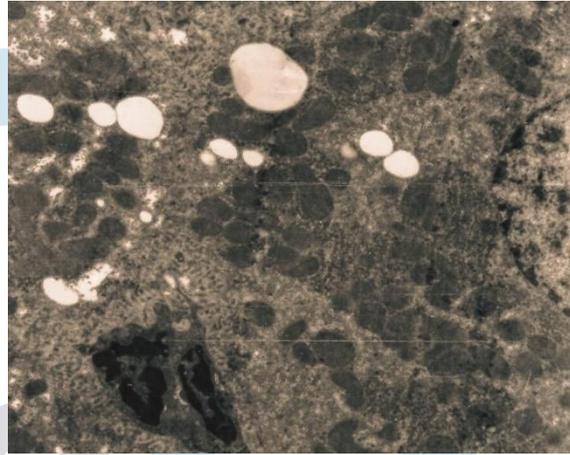


Figure b: T.E.M micrograph of rat liver (Arsenic treated) showing various vacuoles and increased number of mitochondria (x2650)

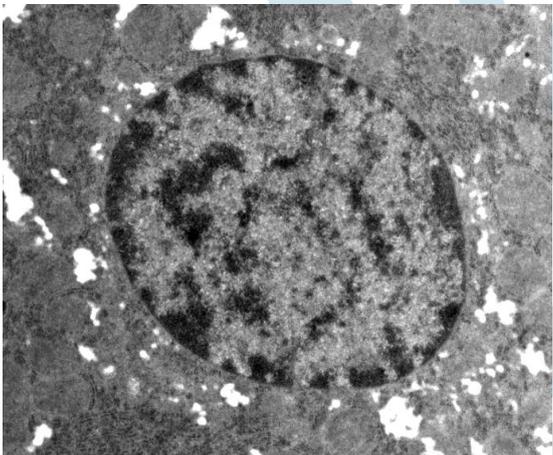


Figure c: T.E.M micrograph of rat liver (Alloxan treated) showing swollen nuclei (x3500).

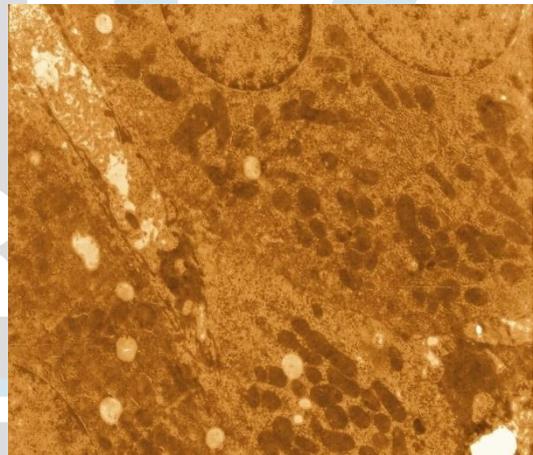


Figure d: T.E.M. micrograph of liver (rat treated with As and alloxan) showing mitochondria (variably sized and shaped) and various vacuoles (x1950)

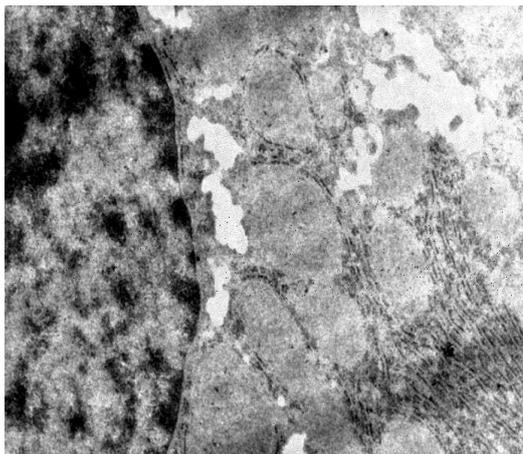


Figure e: T.E.M. micrograph of liver (rat treated with alloxan after insulin treatment) showing improvement in nuclear morphology. (x5600)

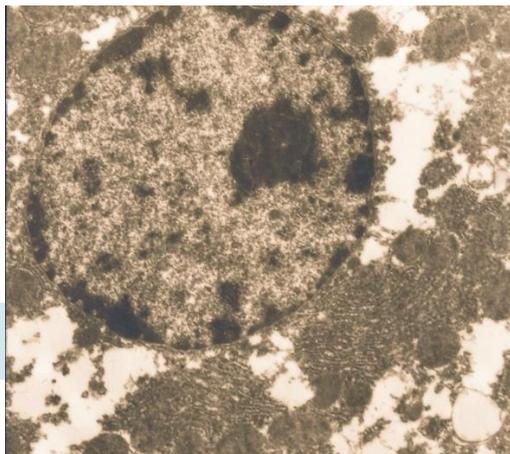


Figure f: T.E.M. micrograph of liver (rat treated with alloxan, fed with As and insulin treated) showing extensive proliferation in E.R (endoplasmic reticulum) (x3400).

Discussion

There are several reports that establish a relationship between hepatotoxicity and arsenic exposure in humans and experimental animals [14, 11, 1]. A few studies show association between arsenic exposure to diabetes status and glucosuria [24, 25].

However, in a study with sample size of 11,319 in the human population from Bangladesh examining correlation among arsenic in dissolved well water, its concentration in urine and occurrence of diabetes mellitus. Insignificant correlation was observed between arsenic contaminated well water and diabetes [26]. Thus there appears to be further need to investigation to the relationship between Arsenic and diabetes mellitus.

T.E.M study on African catfish (*Clarias gariepinus*) liver shows that treating hepatocyte with arsenic (19.2mg/L) causes disruption of intracellular compartments. In the hepatocytes RER (rough endoplasmic reticulum) exhibited cisternae which were fragmented, vesiculated and dilated. On examination alteration in mitochondria and sizes of hepatic cells and their nuclei were seen. In case of the arsenic exposure level being 38.3mg/l the hepatocytes were altered structurally causing decreased level of glycogen in their matrix. There was an elevation in the number of cell organelles (lysosomes and mitochondria) along with size of mitochondria with uneven distribution of these organelles in the cell cytoplasm [27].

In the liver of rat after treating with 0.05mg/l arsenic and 2.5mg/l arsenic the nucleus of hepatic cells was shrunk and chromatin was condensed. In the latter case ER was fragmented. When treated with 5mg/l arsenic rat liver showed similar changes in hepatocytes with notable condensation of its chromatin. There were several vacuoles and granules seen in the hepatocytes along with crenation of its nucleus [28].

In the diabetic rats the glycogen granules were markedly decreased in the hepatocytes. The smooth endoplasmic reticulum (SER) number aggravated whereas the rough endoplasmic reticulum (RER) decreased in number [29]. This study shows the effect on ultrastructure of liver of diabetic rats and when these diabetic rats are treated with arsenic and insulin but there is further need of research on this topic.

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