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Ultrasonographic detection of hepatic tumors in experimental model of chemical induced hepatocarcinogenesis in Wistar Albino rats

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Abstract
In the present study, hepatic tumors were chemically induced in male Wistar Albino rats with Diethylnitrosamine. Hepatic ultrasonography was performed to detect the development of hepatic tumors clinically at the end of treatment period. In sonography, the presence of tumor nodules was appreciated as the indistinct hepatic borders and circumscribed varied echogenicity with distinct hyperechoic borders in the affected portion of liver in contrast to the liver of negative control rats which showed distinct borders, homogenous parenchyma and medium echogenicity.

Keywords: Diethylnitrosamine, rats, echogenicity, ultrasonography

Introduction
Ultrasonography (USG) is routinely performed in dogs and cats to detect chronic hepatic diseases and hepatic tumors. Hepatic ultrasound can non-invasively examine the internal architecture of the liver parenchyma, biliary system, portal and hepatic vascular supply. It is commonly used to detect pathological liver conditions such as cirrhosis and fatty liver disease and it allows assessment of changes in liver echogenicity that correlates with disease (Dias *et al.*, Lessa *et al.* and Song *et al.*) [1-3]. There are very few reports on ultrasonographic examination in lab animals for evaluation of liver pathology. The present study was conducted to analyze the reliability of ultrasonography in detection of hepatic tumors in rats experimentally induced with Diethylnitrosamine (DEN).

Materials and Methods
Experimental animals
The study was conducted on male Wistar Albino rats of 8 weeks age (180-220 g) which were procured from Indian Institute of Science, Bengaluru and were maintained at controlled temperature (23 °C) with daily exposure to a 12:12 light-dark cycle. The protocols for care and management of lab animals were followed as per the rules of CPCSEA, India guidelines and experimentation was done after seeking approval from the Institutional Animal Ethics Committee (IAEC).

Source of DEN
DEN (Product number: N0756 – N-Nitrosodiethylamine liquid, 1 g/ml) used for induction of hepatocarcinogenesis in rats was procured from Merck KGaA, Darmstadt, Germany.

Experimental design
The rats (n=20) were randomly selected and divided into 2 groups of 10 animals each as tabulated below.

Group I	Negative control
Group II	DEN control – DEN at 100 mg/kg intraperitoneally on Day one and subsequently through drinking water at 0.01 % v/v concentration, for 90 days

Ultrasound Imaging

The animals from both control and experiment groups were anaesthetized using ketamine hydrochloride and the abdomen was shaved so as to avoid imaging artifacts during ultrasonographic examination. The ultrasound gel (Aquascan, Zealmax Incorporation, India) was used as conductive medium between the skin and probe. Hitachi Aloka Diagnostic Doppler Ultrasound machine and multi frequency 672CV probe were used for ultrasonographic examination at the Department of Veterinary Gynecology and Obstetrics, Veterinary College, Bengaluru. The ultrasound images were captured at a frequency of 18 – 53 Hz in fundamental two dimensional brightness mode (B-mode). Animals were placed on supine position and the probe was placed caudal to the right costal cartilages so that the beam was directed cranially obtaining multiple scans of liver on USG (Lessa *et al*. and Akshatha) [2, 4].

Results and Discussion

Ultrasonography is a non-invasive, easy to perform, relatively cost effective and easily accessible diagnostic imaging modality used in evaluation of liver. USG can detect changes in the liver and characterize focal lesions (cystic or solid) with high accuracy. Besides being a non- invasive method, it is innocuous, reliable, rapid, reproducible and inexpensive (Akshatha) [4]. Also, ultrasonography allows assessment of changes in liver echogenicity which correlates with disease (Lessa *et al.*) [2].

In the present study, the USG of rats from both groups was done on 90th day of experiment. The livers from control group revealed regular hepatic surface, homogenous liver parenchyma and medium echogenicity (Fig 1) which was in conjunction with the findings of Lessa *et al.* [2] and Song *et al.* [3]. However, the livers from DEN treated rats showed characteristics changes on ultrasonography. They showed indistinct hepatic borders with heterogeneous hepatic parenchyma (Fig 2) and an extreme increase in the size of liver. The presence of tumor nodules was evident by the circumscribed varied echogenicity in the affected portion of liver with distinct hyperechoic borders (Fig 3 & 4). USG revealed the nodules ranging from 0.5 – 4.0 cm. The altered echogenicity in the tumor mass could be attributed to the necrosis in the tumor nodule and the heterogeneous liver parenchyma appearance due to the fibrosis, fatty change, necrosis, vast vascular supply and calcification which corroborates the findings of Song *et al.* [3] and Akshatha, [4]. The peripheral hyperechoic border of tumor nodule could be due to the presence of fibrous tissue in the capsule (Lessa *et al.*) [2].

Comparison of ultrasound findings between livers of control and DEN treated rats

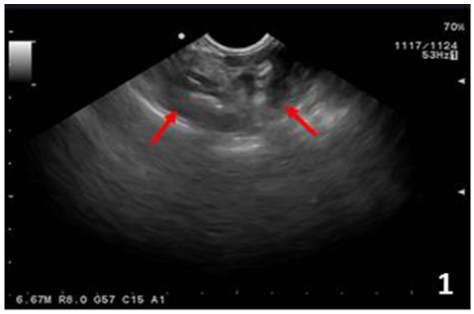

Fig 1: Control – Distinct hepatic borders, homogenous parenchyma and medium echogenicity

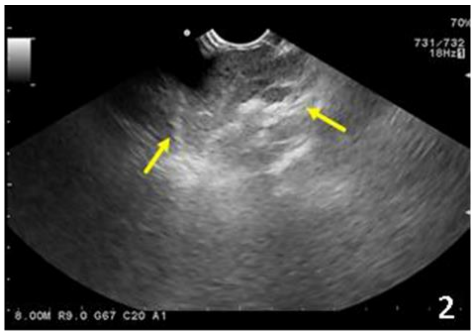

Fig 2: DEN – Indistinct hepatic borders and heterogeneous parenchyma

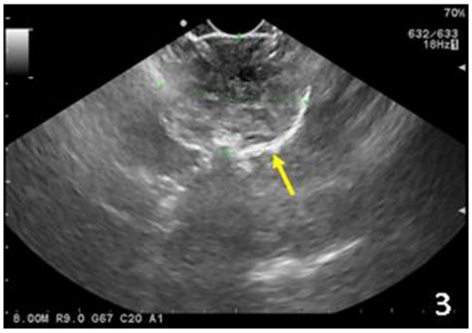

Fig 3: DEN – Tumor nodule with hyperechoic border

Fig 4: DEN – Varied echogenicity within the tumor nodule

Conclusion

Ultrasonography of liver can be used as a reliable qualitative diagnostic modality for confirmation of hepatic tumor induction in murine hepatacarcinogenesis models since it is both feasible and efficient. The implication of this study is to certify that the tumor nodules as small as 0.5 cm can be easily detected using USG. Since it is a non-invasive method, it can be easily employed to detect the formation of tumor nodules without necropsy and decide the further course of study in preclinical trials.

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