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A Study of Histopathogical Examination of Alcohol Induced Hepato-Toxicity in Albino Rats and Protective Effect of *Cuminum cyminum*

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Abstract: ***Aim: To study the histopathology of Alcohol induced Hepato-toxicity in Albino Rats and the protective effect of Cuminum cyminum. Methodolgy: 15 adult albino rats were divided into three groups (n=5). (1) Group one served as experimental control and was fed water extract of cumin at a dose of 100 mg/kg body weight/ day. (2) Group two were exposed to 40 % ethanol continuously by means of intra-gastric feeding at a dose of (v/v) 1ml/ 100gm body weight/ day for 60days. (3)The third group received 40 % ethanol like second group for 60 days and then water extract of cumin at a dose of 100 mg/kg body weight/ day for another 60 days. Liver tissue samples were then collected from all the groups for histopathological study. Results: Liver sections from control rats showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus, nucleolus, central vein and compact arrangement of hepatocytes. In contrast to this, Ethanol caused hydropic changes and necrosis in centrilobular hepatocytes. Congestion of the central vein and sinusoids were seen with acute inflammatory cells infiltrating mainly in the central zone. The midzonal and peripheral hepatocytes showed vacuolization and fatty change (steatosis) which included the intracellular accumulation of neutral fats. In animals treated with Ethanol plus C. cyminum, tissue damage and necrosis were of less extent than the Ethanol Treated Group. Conclusion: Treatment with cumin extract led to a significant attenuation of the liver injury induced by alcohol, in respect of regeneration, prevention and growth of hepatic cells. These effects may be due to the hepatoprotective property of cumin.***

Keywords: Cumin, Heapto-toxicity, Histopathology

1. Introduction

Liver is one of the most important organs of the body involved in a large array of metabolic, excretory, secretory, synthetic, endocrine and exocrine functions etc. which are vital for maintenance and performance of the body. A healthy liver regulates the composition of blood, including the amounts of sugar (glucose), protein, and fat that enter the bloodstream, it also removes bilirubin, ammonia, and other toxins from the blood and processes most of the nutrients absorbed by the intestines during digestion and converts those nutrients into forms that can be used by the body. The liver also stores some nutrients, such as vitamin A, iron, and other minerals, it also produces cholesterol and certain important proteins, such as albumin, clotting factors, chemicals needed to help blood clot etc. Moreover it is also involved in breaks down of (metabolizes) alcohol and many drugs. Thus the maintenance of a healthy liver is of paramount importance.

Because the liver filters toxins from blood, it is especially vulnerable to injury. Hepatic damage caused by chemicals or infectious agents causes distortions of liver metabolic functions (Wolf,1999; Cullen, 2005) and may lead to progressive liver fibrosis and ultimately cirrhosis and liver failure (Anand, 1999).

Ethanol consumption is considered to be a risk factor in the development of liver damage. Alcoholic liver diseases remain one of serious health problems. Alcoholic liver disease is the common consequence of prolong and heavy alcohol intake (Nirwane and Bapat , 2012). The fatal changes in the liver include fatty liver, hepatitis and hepatic cirrhosis (Lieber et al.,2005)

Traditional or herbal drugs have been playing an active role in the health sector worldwide and there is a resurgence of interest in herbal medicines for treatment of various ailments including hepatopathy (Venukumar and Latha 2002). Traditionally plants have been used as medicines against various types of diseases (Gole et al., 1997).

Cuminum cyminum (Cumin) is an ancient herb cultivated across Asia, Africa and other continents. It belongs to the family Apaiaceae. This herb has been known to be used for treatment of various ailments in ancient times and various documentations of its uses are found in various indigenous healing systems, but its therapeutic uses are yet to be validated experimentally worldwide.

Figure: Cumin seeds.

The present study aims to investigate the protective effect of aqueous extract of *Cuminum cyminum* seeds on rat liver damage induced by ethanol.

2. Materials and Methods

Animals

15 albino rats weighting 150g-200g were collected from the Animal House of Department of Zoology, Gauhati University,Guwahat, Assam. Rats were provided food and congenial atmosphere to stay safely. Animals were acclimatized in the laboratory conditions for 30 days after collection. Animals were kept in standard animal cage in a room with 12 hr light/dark cycle at room temperature.

Chemicals

All the chemicals used in the present study were of analytical grade.

Preparation of Extract

Dried cumin seeds were purchased from the local market. Seeds were then washed thoroughly, dried and grinded. After that 200gm of powdered Cumin seeds were dissolved in 500ml of distilled water. The solution was allowed to remain dissolved for 72 hrs after which it was filtered using Whatman's filter paper (No. III), evaporated under vacuum and freeze dried. Resulted light yellowish, dry mass was used to prepare the required dose of Cumin extract for the study.

Experimental Design

A total of 15 animals were taken for the study. Out of the 15 animals, 5animals (2males, 3 females) served as "Control" whereas the other 10 animals were placed in the "Test Groups (1 and 2)". In the test groups all the animals were treated with 40% Ethanol for a period of 60 days.
For the first 10 days each animal received 5ml of 40% ethanol/kg body weight/day, then for the next 20 days they received 10ml of 40% ethanol/kg body weight/day. Lastly for the next 30 days all the animals received a maintenance dose of 10ml of 40% ethanol/kg body weight/day.

After the alcoholic induction, 5 animals from the Test Group-1 were sacrificed on the 61st day i.e. after 24hrs of last dose and liver tissues were collected. The rest 5animals (Test Group-2) were kept for post-alcoholic induction observation.

After 10 days of observation, all the 5 animals were treated with aqueous extract of Cumin at a dose of 100mg/kg body wt. /day for another period of 60 days, after which animals were sacrificed and liver tissues collected for histological study. Two histological slides were prepared from the tissue of each animal from all the three groups.

Groups→ Treatment ↓	Control	Test-1	Test-2
40% Alcohol	---	5ml/kg body wt/day for 10days.	5ml/kg body wt/day for 10days.
40% Alcohol	---	10ml/kg body wt/day for next 50days.	10ml/kg body wt/day for next 50days.
Cumin extract Treated	100mg/kg body wt/day from 70th day to another period of 60 days.	---	100mg/kg body wt/day from 70th day to another period of 60 days.
Sacrifice	On 131st Day.	On 61st Day.	On 131st day.

Histological Study

Liver tissues were dissected out and immediately washed in water and fixed in a fixative i.e. formalin (10%) for 24 hrs. After that tissues were washed and passed through various ascending and descending grades of alcohol for dehydration, tissues were then cleaned in xylene and embedded in paraffin wax. Section of 3µm thickness were prepared and stained using the Hari's Haematoxylin and Eosin staining procedure (Humason,1979).

3. Results and Observations

Behavioural study

A. Alcoholic Induction Phase

1) Control Group

Parameters	1st day of treatment	15th day of treatment	30th day of treatment	45th day of treatment	60th day of treatment
Weight	200gm	200gm	210gm	200gm	210gm
General Examination	Eyes:- Normal Ears:- Normal Body Colour: - Whitish. Limbs:- Normal Tail- Normal	Eyes:- Normal Ears:- Normal Body Colour: - Whitish. Limbs:- Normal Tail- Normal	Eyes:- Normal Ears:- Normal Body Colour: - Whitish. Limbs:- Normal Tail- Normal	Eyes:- Normal Ears:- Normal Body Colour: - Whitish. Limbs:- Normal Tail- Normal	Eyes:- Normal Ears:- Normal Body Colour: - Whitish. Limbs:- Normal Tail- Normal

2) Alcohol Treated Group.

Parameters	1st day of treatment	15th day of treatment	30th day of treatment	45th day of treatment	60th day of treatment
Weight	170gm	155gm	132gm	132gm	110gm
General Examination.	No significant deviation from	Eyes:- A bit dry Ears:- A bit folded	Eyes:- Dry Ears: - Folded half.	Eyes:- Dry Ears: - Folded half.	Eyes:- Dry Ears: - Folded half.

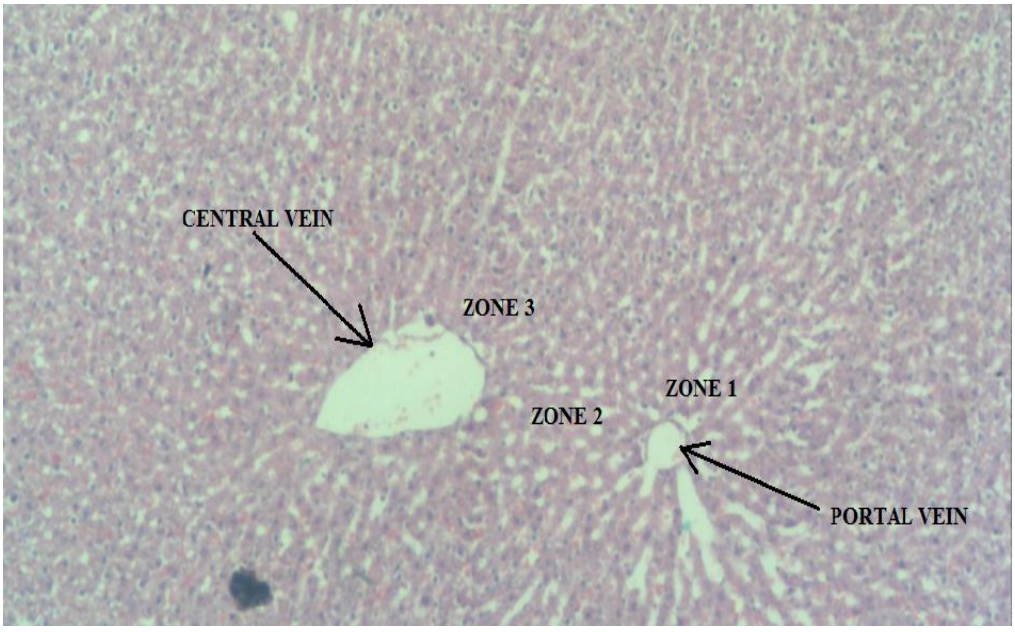
	Control Group.	Body Colour: - Mild greyish white. Limbs:- Normal Tail- Normal	Body Colour: - Pale greyish white. Tail- Fallen	Body Colour: - Greyish white. Tail- Fallen	Body Colour: - Greyish white. Tail- Fallen

3) Post-Alcoholic Induction Group.

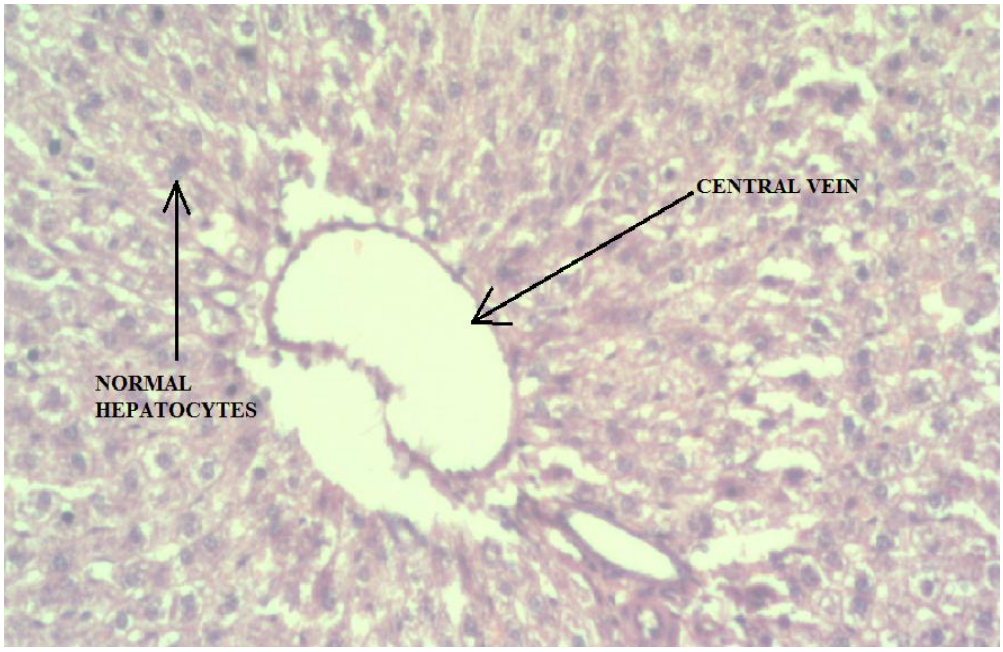
Parameters	1st day of treatment	15th day of treatment	30th day of treatment	45th day of treatment	60th day of treatment
Weight	115gm	117gm	136gm	156gm	171gm
General Examination.	Eyes:- Dry Ears: - Folded half. Body Colour: - Greyish white. Tail- Fallen	Eyes:- Dry Ears: - Folded half. Body Colour: Greyish white. Tail- Fallen	Eyes:- A bit dry Ears:- A bit folded Body Colour: - Mild Greyish white. Limbs:- Normal Tail- Normal	Eyes:- Regaining Normal Condition Ears:- A bit folded Body Colour: - Mild Greyish white. Limbs:- Normal Tail- Normal	Eyes:- Regaining Normal Condition Ears:- Regaining Normal Posture Body Colour: - Fade greyish white. Limbs:- Normal Tail- Normal

Histological Examination

Liver sections from control rats showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus, nucleolus, central vein and compact arrangement of hepatocytes (Fig. 1). In contrast to this, Ethanol caused hydropic changes and necrosis in centrilobular hepatocytes (Fig. 2). Congestion of the central vein and sinusoids were seen with acute inflammatory cells infiltrating mainly in the central zone. The midzonal and peripheral hepatocytes showed vacuolization and fatty change (steatosis) which included the intracellular accumulation of neutral fats. In animals treated with Ethanol plus *C.* Cyminum extract, tissue damage and necrosis were of less extent (Fig. 3) than the Ethanol treated group.

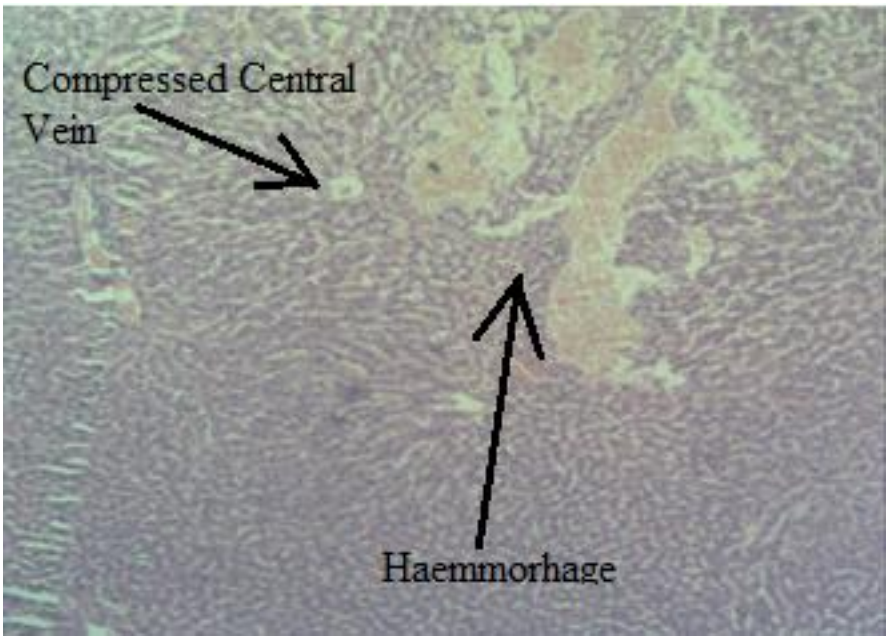


A.

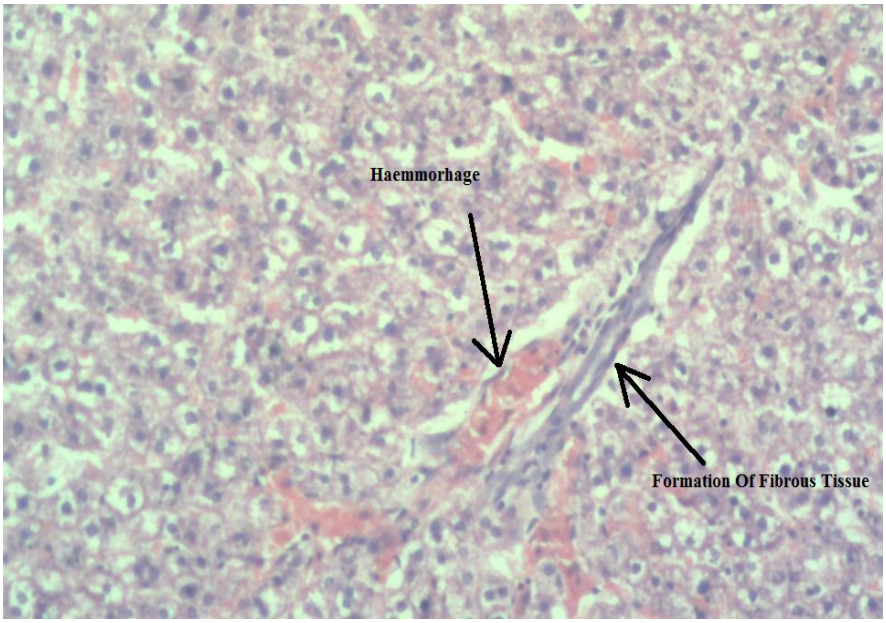


B.

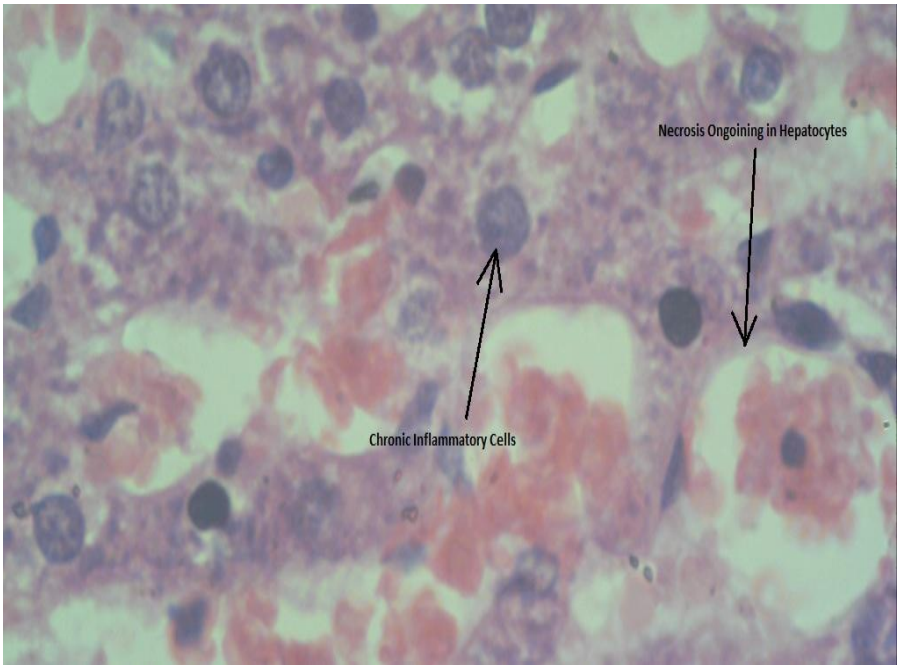
Figure 1: Sections of paraffin-embedded liver tissue of Control Group, stained with hematoxylin–eosin. [(A).4x magnification. (B). 10x magnification] Liver is divided histologically into lobules. The center of the lobule is the central vein. At the periphery of the lobule are portal triads. Functionally, the liver can be divided into three zones, based upon oxygen supply. Zone 1 encircles the portal tracts where the oxygenated blood from hepatic arteries enters. Zone 3 is located around central veins, where oxygenation is poor. Zone 2 is located in between.



A.

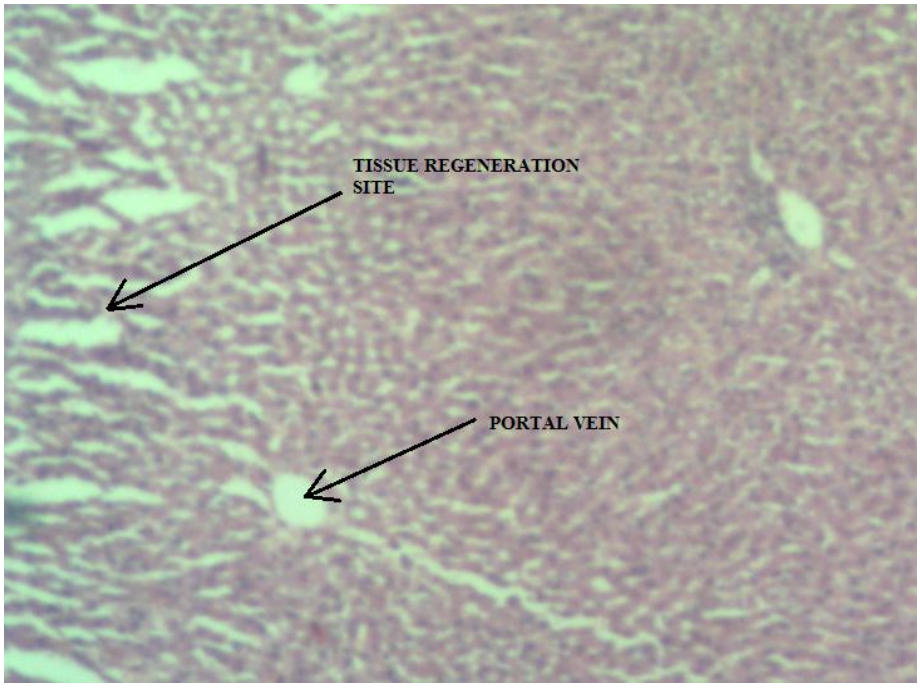


B.

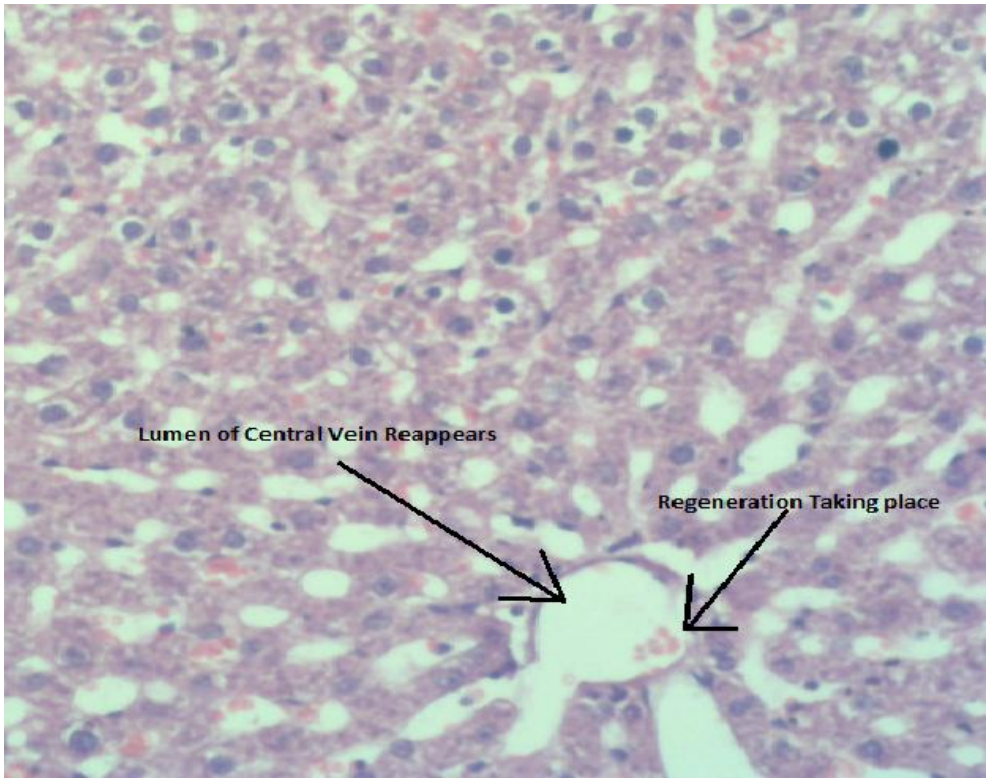


C.

Figure 2: Sections of paraffin-embedded liver tissue of Ethanol Treated Group were stained with hematoxylin–eosin. [(A).4x magnification. (B). 10x magnification. (C) 40x magnification.].



A



B.

Figure 3: Sections of paraffin-embedded liver tissue of Post-Alcoholic Induction + Cumin Treated Group were stained with hematoxylin–eosin. [(A).4x magnification.B. 10x magnification].

4. Conclusion

On the basis of results it is seen that pre-treatment with cumin extract led to a significant attenuation of the liver injury induced by alcohol, in respect of regeneration, prevention and growth of hepatic cells. Moreover certain behavioural changes observed in the alcohol treated group seem to regenerate after treatment with Cumin extract. These effects may be due to the hepatoprotective property and free radical scavenging mechanism of different constituents of the Cumin extract.

Liver diseases remain one of the serious health problems. Conventional or synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. So there is a world-wide trend presently to go back to natural products of herbal origins that are in use for the treatment of liver ailments including hepatic parenchymal regeneration.

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