

Ultrastructural alteration in liver of diabetic mice and repaired by aqueous leaf extract of *Carica papaya*

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Abstract: A significant role of *Carica papaya* aqueous leaf extract was observed in the liver of hyperglycemic swiss albino mice under surface electron microscopic examination. Diabetic mice treated with aqueous leaf extract of *Carica papaya* for 56 days at one time dose of 150 mg/kg body weight for orally daily. The total dose of Alloxan monohydrate ($450 \text{ mg kg}^{-1} \text{ bw}^{-1}$) was administrated in three injections at interval of 48 hrs ($150 \text{ mg kg}^{-1} \text{ bw}^{-1}$) each time. Mice with blood glucose level of greater than 200 mg/dl were considered diabetic and were selected for study. Remarkable changes that occurred in the liver of diabetic mice verses control mice. These changes include degenerative alteration represented by disorganization of the hepatic cords, vacuolated hepatocytes, cytoplasmic vacuolization and picnotic nuclei of hepatocytes and inflammatory cell infiltration.

Index Terms – Hyperglycemic, Alloxan monohydrate, *Carica papaya* leaf, Aqueous extract, Scanning electron microscope

1. INTRODUCTION

Diabetes mellitus is a diverse metabolic endocrine disorder. It is caused by the deficiency or inadequate production of insulin by Pancreas that results in increase or decrease concentration of glucose in the peripheral blood (Altan, 2013). Diabetes is an ongoing epidemic, in the present day due to increased sedentary habits urbanization and reckless consumption of junk food, more numbers of people are suffering from diabetes. In recent years, WHO opined that this may increase to 440 million by 2045 (WHO, 2017). In the same year in Indian scenario, it is diagnosed to have nearly 65 million diabetics, followed by Sri Lanka which has over 1.16 million diabetics (Bandera, 2016).

Diabetes occurs, if the fasting blood glucose level rises from 70 to 99 mg/dl or more, or a random blood glucose test shows a level of over 200 mg/dl or more. Diabetes is associated with premature mortality, predominantly through atherosclerotic vascular disease and micro-vascular complications which affect the small blood vessels in the eye, kidney and nerves (Azuma *et.al*, 2017). So the present study was carried out on the Diabetic mice treated with aqueous leaf extract of *Carica papaya* to see the remedial option for the diabetic sufferers in developing countries.

2. MATERIALS & METHODS

2.1 Plant material:

Fresh leaves of *Carica papaya* were collected from plants grown in the University Department of Botany campus, of T. M. Bhagalpur University, Bhagalpur. The leaves were shade dried and powder with help grinder and were kept in air tight container separately until the time of use.

2.2. Preparation of aqueous extract:

Powder of *Carica papaya* leaves (100g) was taken and 200 ml of distilled water added and boiled then filtered off by using whatman's filter paper No.41. The final concentration of the extract was 150mg/ml. The filtrate obtained served as crude extract and administered orally to the experimental animals at the concentration of 150mg/kg body weight per day for 8 weeks (Pochapski *et.al*, 2011).

2.3. Experimental animals and induction of diabetes:

Adult swiss albino mice (25 -30g) bred in the Animal house, University Department of Zoology Science campus, T. M. Bhagalpur University, Bhagalpur were used in this study. The animals were housed in polypropylene cages under controlled condition of 12 hrs light/dark cycle and at 24°C. They were maintained on standard diet and water ad libitum (Zarrow *et.al*, 1964).

Diabetes was induced in mice by intraperitoneal injection of 150mg/kg body weight of Alloxan monohydrate (5% w/v) freshly dissolved in physiological saline immediately before use (Dunn and Meletchie, 1943).

The diabetic state was confirmed after 48 hrs of Alloxan monohydrate injection. The animals which presented blood glucose level above 200 mg/dl as well as with clinical signs of polydipsia, polyuria and polyphagia were selected for the experiments (Jennigs *et.al*, 1983 and Sacs, 1997).

Treatment:

Test animals were divided into following groups containing 6 mice in each group.

Group 1-Control

Group 2-Diabetic Control

Group 3-Diabetic fed with aqueous extract of *Carica papaya* leaves.

The total experimental protocol was maintained for 56 days (8 weeks) after induction of diabetic as per method suggested by (Zarrow *et.al*, 1964).

2.4. Micro anatomical process for Scanning Electron Microscopy (SEM):

Control and treated swiss albino mice (*Mus musculus*) were sacrificed under chloroforms anesthesia and the liver were removed immediately after dissection. After those liver were transversely cut and exposed the tissues were rinsed in Phosphate buffer 5 - 10 minutes along with Tween solution for removal of mucous from the tissue. After rinsing in buffer, the tissues were fixed in 2.5% gluteraldehyde for 24 hr at 4 degree centigrade. After fixation, the tissues were removed, rinsed in buffer and post-fixed in 1% OSO_4 for 2 hr and again rinsed in 0.1M Phosphate buffer and dehydrated in graded Acetone followed by

Amyle Acetate. These tissues were dried by Critical Point Method with liquid Carbon Monoxide. The tissues were cemented to metal stub and coated with gold to a thickness of approximately 20 nm. The tissues were examined under Hitachi S-530 SEM (Bob Hafner, 2017). The entire process was done in University of Burdwan, Burdwan, West Bengal.

Ultrahistoarchitecture of liver was observed in control, diabetic and *Carica papaya* leaf extract for 56 days treated mice respectively by scanning electron microscope.

3. RESULT AND DISCUSSION

Ultrastructure of liver among different test group animals (Group-1, Group-2 and Group-3) has shown in Fig. 1, 2 and 3 respectively.

Observations:

Group 1 - Scanning electron micrograph of control mice liver examination revealed few hepatocytes containing small pores (Fig.1) and conjoined sinunoids.

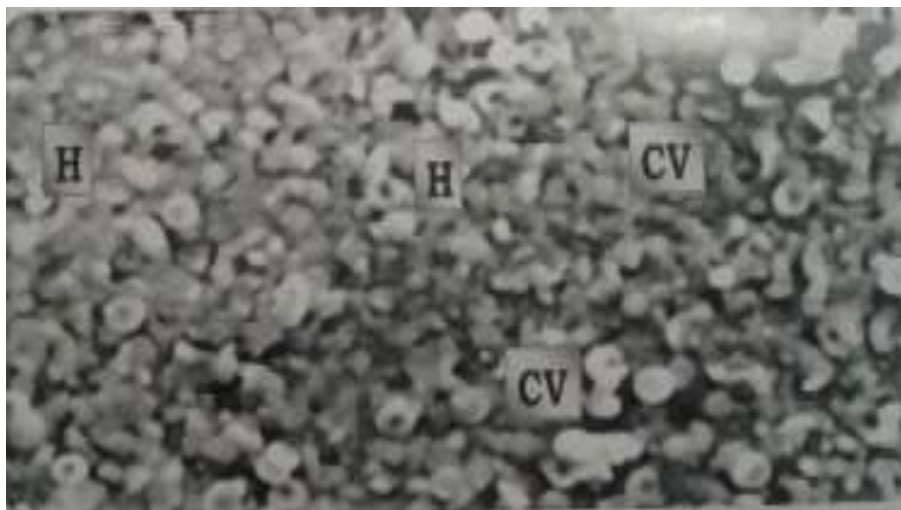


Fig. 1: Scanning electron micrographs of liver of control mice (X 350) showing normal hepatic cord around Central vein (CV), Hepatocytes (H) X 350

Group 2 - Microscopic examination of the diabetic mice observed some remarkable changes versus the control mice. These changes include degenerative alterations represented by disorganization of the hepatic cords, vacuolated hepatocytes and cytoplasmic vacuolisation and pyknotic nuclei of hepatocytes inflammatory cell infiltration (Fig. 2). Areas of hypatic necrosis appeared in some regions. Scanning electron microscopic examination showed a plenty of lipid droplets within the hepatocytes. Furthermore, damaged blood sinusoids and hemorrhage of erythrocytes between hepatocytes and incide lumen were detected (Fig. 2).

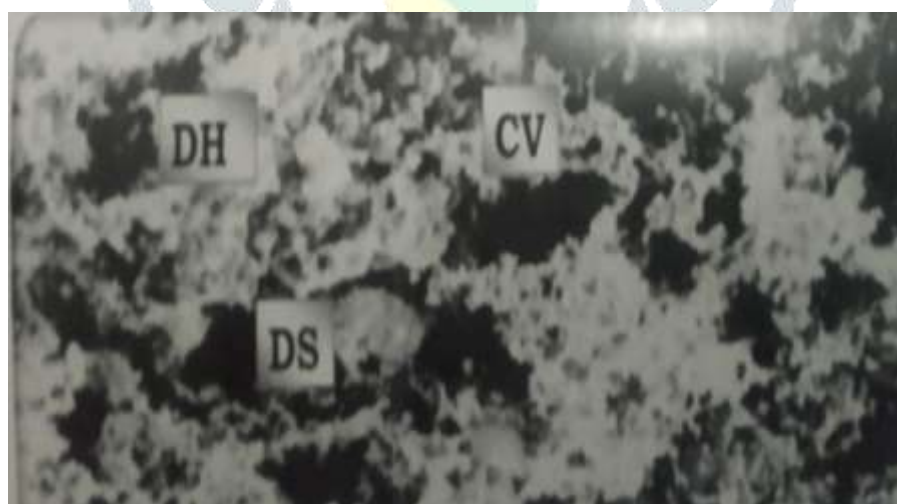


Fig. 2: Scanning electron micrographs of liver of diabetic mice (X 1100) showing degenerating Hepatocytes (DH), Damaged Blood Sinusoids (DS) spread inside hepatocytes X 1100

Group 3 - Surface electron microscopic examination of the liver of diabetic mice treated with *Carica papaya* aqueous leaf extract for 56 days revealed remarkable improvement of hepatic tissue versus the diabetic mice. This was represented by intact blood Sinusoids and hepatocytes. Except a few hepatocytes around the central vein which still had some more cytoplasmic vacuoles. Neither erythrocytes hemorrhage nor were inflammatory cells in filtrations observed (Fig. 3).

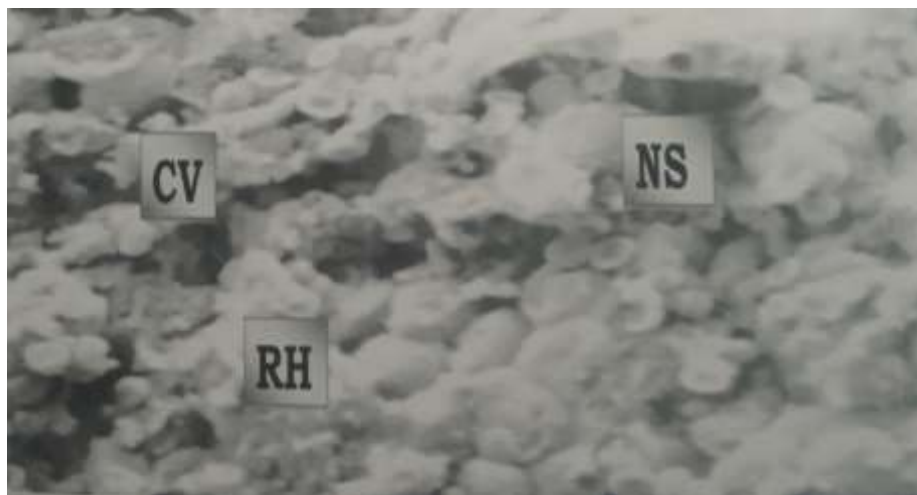


Fig. 3: Scanning electron micrographs of liver treated mice (X 900) in *Carica papaya* leaf aqueous extract (200 mg) terated diabetic mice, showing regenerating Hepatocytes (RH) and blood Sinusoids (NS). New Hepatocytes around the central vein (CV) have seen X 900.

Phytochemical analysis of *Carica papaya* leaves shows the presence of alkaloids, glycosides, minerals, vitamins and many others compounds (Juarez-Rajop *et.al*, 2012). It has been reported the effect of alkaloids, flavonoids and glycoside component as a protective properties of liver as an antioxidant (Olsuki *et.al*, 2010).

The varied composition found in this leaf extract assigns to its antioxidant property. This property of *Carica papaya* extract may also be, because of its other properties like anti inflammatory property which may prevent inflammatory liver damage, immuno modulatory property and antioxidant property (Aларcon-Aguilara *et.al*, 1998). Thereby reducing the oxidative stress imposed by the chemicals (Alloxan monohydrate); this antioxidant mechanism seems to be important as *Carica papaya* leaves has been shown to reduce oxidative stress (Indran *et.al*, 2015).

4. CONCLUSION

In conclusion, in light of the beneficial hepato-protective effects of aqueous leaf exattract of *Carica papaya* detected in the current investigation. It is advisable to widen the scale of its use, after further purification procedure, for patients at high risk of diabetes mellitus in a trial to alleviate the diabetic undesirable hepatic hazards.

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