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Role of EGF Secreted by Submandibular Gland in Acetaminophen induced Acute Liver Failure

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ABSTRACT

Epidermal growth factor (EGF) is most extensive factor secreted by submandibular gland and it has important physiological roles like cell proliferation, regulates tissue differentiation and modulates organogenesis. This study was carried out to investigate potential effect of submandibular gland secreted epidermal growth factor (EGF) on acetaminophen induced acute liver damage. Acetaminophen (APAP) overdose causes acute liver injury or even death in both humans and experimental animals. For this study mice were divided into three groups viz- i. Control ii. Control + APAP iii. Sialoadenectomy (submandibular gland removed) +APAP group. Mice were given acetaminophen (300 mg/kg; orally) to induce acute liver injury. After 1, 3,6,12, 96 hr of APAP administration. histology of liver and SGOT , SGPT from blood was studied. Acetaminophen significantly increased the levels of SGOT, SGPT and also caused severe necrotic changes in liver tissue. In group III i.e. in Sialoadenectomy+APAP there was significant increase in both the enzymes i.e. SGOT and SGPT as compared to group II i. e. Control+APAP and as time progressed i.e. from 1,3,6,12,96 hr there was significant increase in both enzymes and show maximum value in 96 hr post treatment. Our result indicate that submandibular gland EGF have role in proliferation of hepatocytes.

Keywords: EGF, APAP, Sialoadenectomy etc.

INTRODUCTION

The liver is one of the most vital organ that functions as a center for metabolism of nutrients and excretion of waste materials. The liver handles the metabolism and excretion of drugs from the body their by providing protection against foreign substances by detoxifying and eliminating them. Paracetamol (acetaminophen) is a widely used antipyretic and analgesic which produces acute liver damage if overdoses are consumed. Paracetamol is mainly metabolized in liver to excretable glucuronide and sulphate conjugates (Nanji *et al.,* 2002 ; Jollow *et al,*. 1974). Paracetamol can extensively metabolized by the liver via three main pathways; sulfonation, glucronidation and oxidation (Mitchell *et al.,* 1974). The first two pathways are quantitatively more important than the last one, but the oxidative pathway is the culprit as far as toxicity is concerned (Jollow *et al.,* 1974). Oxidation of paracetamol occurs in the

hepatic microsomes and is primarily catalyzed by cytochrome P-450 (Potter *et al.,* 1973). However, the heaptotoxicity of paracetamol has been attributed to the formation of toxic metabolites when a part of paracetamol is activated by hepatic cytochrome P-450 (Savides and Oehme,1983) to a highly reactive metabolite N-acetyl -P-benzoquinone imine (NAPQI) (Vermeulen *et al*.,1992). NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid (Moore *et al*.,1985). However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or -SH group of protein and alters the homeostasis of calcium after depleting GSH. It has been established that a hepatotoxic dose of paracetamol depletes the endogenous glutathione level to below a threshold value <20% of control), therefore permitting interaction of NAPQI with cell macromolecule (Potter and Himson., 1986).

Submandibular gland secretes several biologically active peptides and hormones, including epidermal growth factor (EGF), Nerve growth factor (NGF), mesodermal growth factor (MGF) and hepatocytes growth factors (HGF) (Amano, and Iseki, 2001). Excision of these glands (Sialoadenectomy) has been used as an experimental model to examine the biological functions of some of these factors. Out of the different growth factors secreted by the submandibular gland EGF is most extensively studied factor because it has important physiologic roles like cell proliferation, regulates tissue differentiation and modulates organogenesis (Yan, *et al.,* 1998)

Previously many scientists have studied the effect of submandibular gland secreted growth factor on growth and development of different organs (Pillai and Walvekar, 2002, 2005, 2007, 2009, Walvekar *et al*., 2010, 2011). This present study was designed to determine the effect of submandibular gland secreted growth factors on liver injury after paracetamol induced acute liver damage in mice by studying parameters like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and histology of liver.

MATERIAL AND METHODS

Animal:

One month Swiss albino mice (*Mus musculus*) weighing 18-22gm were used for the present study. Animals were housed in departmental animal house (1825/PO/ EReBi /S/15/CPC EA) in separate cages under proper conditionof a 12:12 hr L:D cycle. They had free access to standard rodent pelleted diet (Nutrinix std- 1020, mfg. by.- Nutrivet life sciences, Pune) and water was given *ad libitum.*

Experimental design: Mice were divided into three groups in each group ten.

1. **Control group**: The controls were sham operated i.e. the procedure identical to sialoadenectomy except that the glands were not removed and received distilled water. Operated mice were maintained with proper care up to the age of one month.
2. **Control + APAP group**: The controls were sham operated i.e. the procedure identical to sialoadenectomy except that the glands were not removed and after seven days animals orally received paracetamol (300mg/kg body weight) (Victor, *et al*., 2011)
3. **Sialoadenectomised +APAP group**: In the 20 days old male mice submandibular glands were removed (sialoadenectomised) and after seven days animals orally received paracetamol (300mg/kg body weight).
4. Mice from all groups were killed by cervical dislocation after 1,3.6.12,96 hrs after APAP adminisration. Blood was collected by cardiac puncture and liver was dissected out and used for biochemical and histological study.

Chemicals:

Acetaminophen tablet-500mg, SGOT, SGPT Kit (Coral clinical system)

Sample collection:

Blood sample of each group having different time interval was collected separately into sterilized dry centrifuge tubes and allowed to coagulate for 30 min and then centrifugated at 3000 rpm at 10min for separation of serum. Serum was used for biochemical analysis such as SGOT, SGPT.

Histological examination (Harris., 1900)

For histological study the liver was fixed in 10% Formaldehyde for 24 hours. The glands were washed in running tap water for 24 hours, dehydrated through alcoholic grades, cleared in xylene and embedded in paraffin wax. The sections were cut at a thickness of 5µ and stained with Hematoxylene and Eosin (H/E).

Statistical Analysis:

All values were expressed as mean ±SD. Statistical analysis was carried out by one-way ANOVA, Turkey's HSD test.

RESULTS

Time-course effect of acetaminophen on SGOT and SGPT :

To investigate the time-course effect on acetaminophen induced liver damage in sialoadenectomised mice; we measured the SGOT and SGPT levels at different time intervals.

Table 1: Effect of Submandibular gland secreted growth factors on liver injury after Acetaminophen induced acute liver failure on Serum Glutamate Oxaloacetate Transaminase (SGOT) (SGOT activity in U/L). Values are mean ± S.D. (Numbers in parenthesis denotes number of animals).

Group	Time interval in hour				
	1	3	6	12	96
Control (5)	35.2±6.6483				
Control+ APAP(5)	34±1.5811	39.8±1.3038	69.4±1.1402	97.8±1.4832	176.92±1.5912
Sialo+ APAP(5)	61.8±1.7889	80.746±1.9203	127.608±1.1438	132.17±1.752	201.6±2.881

Table 2: Effect of Submandibular gland secreted growth factors on liver injury after Acetaminophen induced acute liver failure on Serum Glutamate Pyruvate Transaminase (SGPT) (SGPT activity in U/L). Values

Group	Time interval in hour				
	1	3	6	12	96
Control (5)	19.174±4.9388				
Control+ APAP (5)	17.892±1.8864	27.8±1.3038	52.2±1.3038	62.6±1.8166	152.066±0.9256
Sialo+ APAP (5)	73±2.2361	97.908±1.1456	109.466±1.1206	128.808±1.2978	181±1.5811

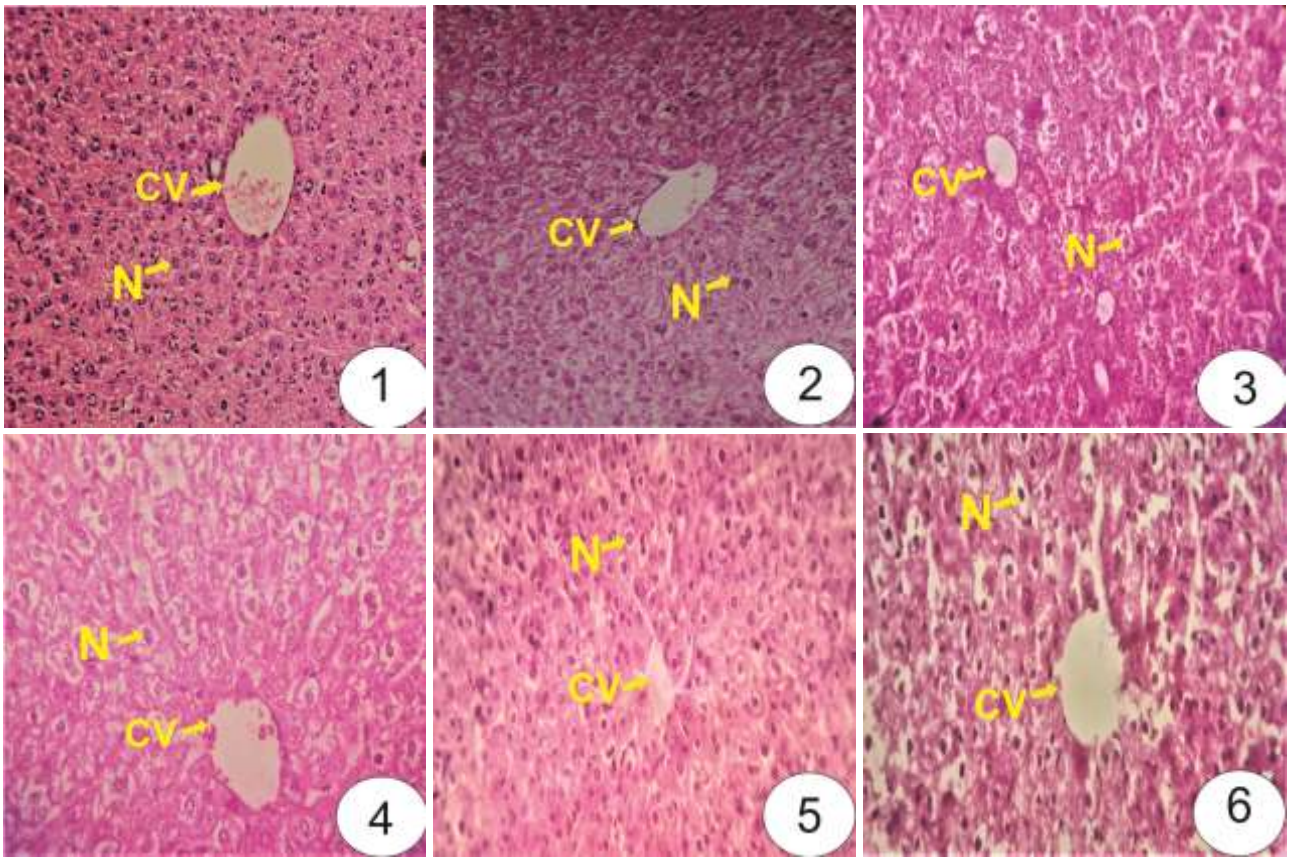

Plate 1: Histopathological changes in liver of Control and +APAP mice, All the sections stained by HE (X 400).
Fig.1: Control mice liver shows normal lobular architecture, central vein and normal arrangement of hepatic cords.
Fig. 2 to 6: Control +APAP mice live shows destroyed architecture, micro and macro vesicular fatty changes.
Captions :CV- Central Vein **N-** Nucleus.

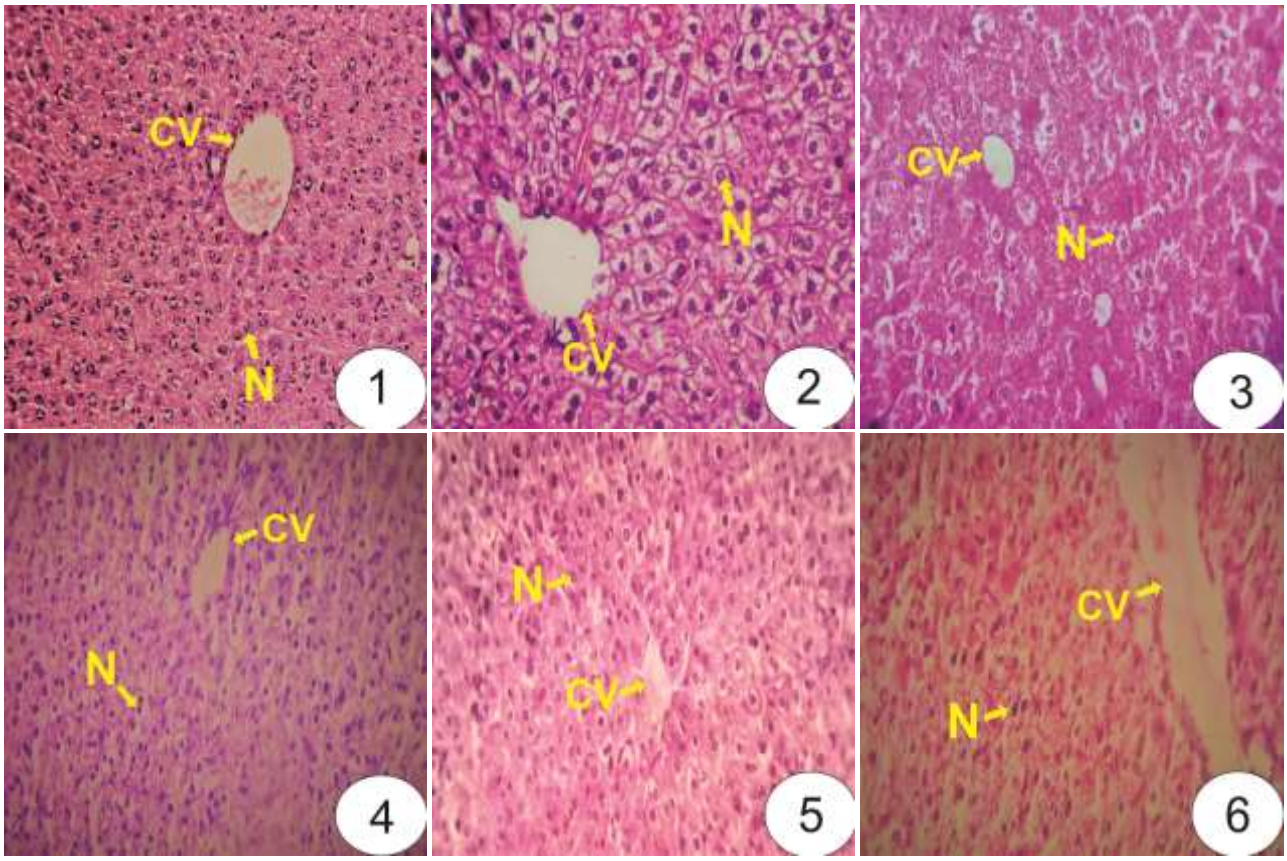

Plate 1: Histopathological changes in liver of Control and Sialoadenectomised +APAP mice, All the sections stained by HE (X 400). **Fig.1:** Control mice liver shows normal lobular architecture, central vein and normal arrangement of hepatic cords. **Fig. 2 to 6:** Sialoadenectomised +APAP mice live shows severe necrotic.
Captions :CV- Central Vein **N-** Nucleus.

In group I, level of SGOT and SGPT was 35.2±6.6483 and 19.174±4.9388. While in group II i.e. in control+APAP., the level of both enzymes was significantly and progressively increased at different time interval i.e 1, 3, 6, 12, 96 hr of post treatment and the increase. SGOT and SGPT levels reached their maximum in Control+ APAP group in 96 hr post treatment. In group III i.e in Sialoadenectomy+APAP there was significant increase in both the enzymes as compared to group II and as time progressed i.e. from 1,3,6,12,96 hr there was significant increase in SGOT and SGPT and show maximum value in 96 hr post treatment.

Time-course effect of acetaminophen on Histology of liver:

Control mice liver has normal histology with normal hepatocellular architecture with Central vein (Plate No. I Fig 1). Cytoplasm of hepatocytes stained with pink in color while prominent nuclei appear violet in color. The cells have well defined cell borders, are polygonal and are arranged in sheets. Liver sinusoids were not dilated. In group II mice hepatocytes showed (Plate No. I) irregular size, shape and orientation while nucleus was enlarged, displaced and vacuolated. Moderate macrovesicular fatty degeneration of liver with dilated sinusoids was observed. An increase in damage after treatment of acetaminophen with different time interval (Plate No. I, Fig. 2 to 6). In group III mice hepatocytes show more damage as cloudy swelling of fat droplets, very severely degenerated hepatocytes, very severely congested sinusoids and damaged central vein, severe necrotic changes are observed (Plate no. II, Fig no 2 to 6) and these necrotic changes was more as time interval increases.

DISSCUSSION

We have removed the submandibular gland of mice at the age of 20th day because at that age the submandibular gland starts to secrete the growth factors. The acute liver failure in mice after a one week sialoadenec-

tomy was done with APAP and then effect of APAP on group II and group III by various parameters such as SGOT, SGPT and histology of liver was studied. out of the different growth factors secreted by submandibular gland, the receptors of EGF i.e. HGF is highly expressed in the liver (Naldini *et al*., 1991), and it was found to be essential for liver development. Previously nobody had studied the sialoadenectomy effect on liver damage after paracetamol induced acute liver failure. Hepatocytes are the main component that regulates various metabolic activities of liver. Distortion of this organ will result in disorder of body metabolism. In the assessment of liver damage certain biomarkers of hepatotoxicity are measured and one of such biomarkers are enzyme levels such as SGOT and SGPT because liver damage arising from necrosis or membrane damage normally releases the enzymes into circulation; therefore, measurement of these enzymes in serum gives an indication of the health status of the liver. High levels of AST indicate liver damage, as that due to viral hepatitis as well as cardiac infarction and muscle injury. ALT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. It is known that an increase in the enzymatic activity of ALT and AST in the serum directly reflects a major permeability or cell rupture, and thus ALT is more specific to the liver, and is thus a better parameter for detecting liver injury (Benjamin, 1978 and Wittwer and Bouhmwald, 1986). An increase in AST and ALT, a hepato-specific enzyme that is principally found in the cytoplasm in the rats following administration of a hepatotoxin is attributed to the increased release of enzymes from the damaged liver parenchymal cells (Benjamin,1978, Ringler and Dabich, 1979; Singh, 1980).

The HE staining showed loss of structural integrity and severe necrotic changes in hepatocytes of both groups i.e. group II and group III but comparatively more damage observed in group III i.e. sialoadenectomy +APAP; due to absence of EGF secreted by submandibular gland. Due to that the growth, proliferation and regeneration in liver may get affected/decreased.

Similar results were obtained in biochemical parameters, the level of both enzymes i.e SGOT and SGPT was increased significantly in group II and group III but comparatively more in group III i.e. sialoadenectomy+APAP. The decrease of these enzymes in circulation indicates the healthy status of liver but due to acute liver damage these enzymes increases in circulation.

CONCLUSION

Thus, our results indicate that sialoadenectomy not only reduces growth, proliferation of hepatocytes but also reduces regeneration of hepatocytes, due to absence of EGF in liver injury.

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