The Early Phase of Acute Paracetamol-Induced Liver Injury in Albino Rats.

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ABSTRACT

Liver plays a central role in transforming, clearing the chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents when taken in overdoses and sometimes even when administered within therapeutic ranges may injure this organ. The aim of our study was to investigate the relationship between liver antioxidant capacity and hepatic injury in the early phase of acute paracetamol intoxication in albino rat.A total number of 24 albino rats were included in the study and was divided into 2 groups. Group-I: Control group (n=6) and Group-II: Paracetamol treated group(n=18) in a dose of 2gm/kg b.wt. At the end of the experiment rats were sacrificed by cervical dislocation 6, 24, and 48 hours after paracetamol administration. Oxidative stress parameters were determined in blood samples spectrophotometrically. Plasma malondialdehyde was significantly increased 6 hrs after paracetamol administration in comparison with control group. After this period MDA level showed a gradual increase and reached its highest value at 48 hours after treament. Paracetamol induced a significant reduction in serum superoxide dismutase (SOD) activity at 6 hrs first. Our data showed that liver antioxidant capacity increases in first 6 hrs of paracetamol-induced liver injury. SOD activity in the serum was significantly lower in paracetamol group in comparison with control group. Similar activities were measured 24 and 48 hours after paracetamol administration. Serum activities of aminotransferases and alkaline phosphatase were significantly increased after paracetamol administration at all time intervals in comparison with control group.These effects are maintained for the next 48 hrs. On the other hand, antioxidant capacity of hepatocytes is increased within 6 hrs, at first, According to these findings, it can be estimated that antioxidant capacity should be administered as early as possible after paracetamol intoxication.

Keywords: Paracetamol, Oxidative stress,Acute hepatotoxicity, Albino rat, Antioxidant

INTRODUCTION

Liver plays an important role in regulation of physiological processes, involved in several vital functions such as storage, secretion and metabolism. It also detoxifies a variety of drugs and xenobiotics and secretes bile that has an important role in digestion. Paracetmol is probably the most versatile and widely used analgesic and antipyretic drug. It is well-known that this drug exerts hepatotoxic effects in a dose-dependent manner. In overdose (over 2 g/day paracetamol), centrilobular hepatic necrosis is recognized as a dominant morphologic alteration. However, pathogenesis of centrilobular hepatic necrosis is not completely understood. Metabolic activation of paracetamol is considered to be a major mechanism of its hepatotoxicity. Namely, it was shown that paracetamol is metabolically activated in the liver by cytochrome P450 to form a reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) that covalently binds to protein. NAPQI is detoxified in the liver by glutathione (GSH) to form a paracetamol–GSH conjugate. When NAPQI production exceeds GSH detoxification capacity,

NAPQI covalently binds to various cell proteins to form inactive conjugates. These conjugates can lead to irreversible hepatic cell injury and liver necrosis by various mechanisms.

Besides, NAPQI binds to sulfhydryl groups of enzymes and in that way may contribute to metabolic alterations in the liver. Migration and activation of neutrophils in the liver, production of cytokines (tumor necrosis factor-a /TNF-a/, interferon-c /IFN-c/, chemokines) and platelet activating factor (PAF) are additional mechanisms that have been reported to be important in the development of paracetamol hepatotoxicity. The precise role of oxidative stress in paracetamol-induced liver injury is not fully described. It has been shown that binding of NAPQI to GSH sulfhydryl groups results in reduction of hepatic antioxidative capacity. Also, it has been postulated that NAPQI can lead to direct oxidative damage of numerous cell components. On the other hand, several studies indicate that increase in serum transaminase activity occurs prior to reactive oxygen species formation, thus suggesting that oxidative stress is not a primary mechanism of paracetamol-induced hepatotoxicity. Some investigations even suggest that NAPQI demonstrates antioxidative properties. In addition, changes in antioxidant capacity of hepatocytes, as well as their role in acute paracetamol-induced liver injury are even more blurred. The precise role and time-course of oxidative stress could have therapeutic implications, since timely applied antioxidant therapy could possibly alter the outcome of paracetamol intoxication. Therefore, the aim of our study was to investigate the dynamics of oxidative stress and relationship between liver antioxidant capacity and liver injury in the early phase of acute paracetamol intoxication in albino rat

MATERIALS AND METHODS

ANIMAL -

The present study was conducted on Albino rats after approval from the Institutional Ethics Committee. Albino rats of Charles Foster strain of either sex weighing between 180-250g were procured from the Central Animal House, Institute of Medical Sciences, Banaras Hindu University, Varanasi.

All the animals were kept in colony cages at an amibient temperature of 25 ± 2^0C with 45-55% relative humidity and 10 : 12 h. light and dark cycle. Animals were kept on standard rodent diet and water *ad libitum.* All the experimental animals were acclimatized in the department for 3 days. Principles of laboratory animal care (NIH Publication No. 86-23 revised 1955) guidelines were followed.

CHEMICALS AND ANALYSING KITS –

Chemicals and analysing kits of different parameters of liver like SGOT ,SGPT ,ALP, Bilirubin Protien,were procured from Avicon diagnostics,Varanasi. Chemicals for MDA and SOD were purchased from Gupta Enterprises, Varanasi.

ASSAY PROCEDURE-

The serum samples were subjected to assay for hepatic marker enzymes such has Aspartate transaminase (AST) Alanine transaminase (ALT) and Alkaline phosphatase (ALP). Activities of AST and ALT were assayed according to the 2-4 DNPH method. Values are expressed as IU/dl ALP activity was measured using the method of Kind and King (1954) and results are expressed as K.A. units/L, bilirubin (Mallay and Evelyn, 1937) and protein (Lowry *et al.*, 1951).The LPO in the serum was determined by the method of Ohkawa .The SOD level in serum and was measured by using modified kakkar method.

EXPERIMENTAL PROCEDURE-

A total number of 24 rats were included in the study and was divided into 2 groups.

Group-I : Control group (n=6)

Group-II : Paracetamol treated group(n=18) in a dose of 2gm/kg b.wt.

At the end of the experiment rats were sacrificed by cervical dislocation 6, 24, and 48 hours after paracetamol administration.

Animals from control and paracetamol treated group were sacrificed at all time intervals. Blood samples for determining parameters of oxidative stress were collected from the right side of the heart. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters.The liver was immediately isolated and washed with normal saline, blotted with filter paper and weighed. Liver tissue homogenate (LTH) was prepared in normal saline and used for estimation of endogenous marker enzymes and biological antioxidants superoxide dismutase (SOD) activities.

HISTOPATHOLOGICAL EXAMINATION-

Liver pieces were preserved in 10% formaldehyde solution for histopathological study. The pieces of liver were processed and embedded in paraffin wax after paraffin embedding and block making, serial sections of 5m thicknesses were made, stained with Haematoxylin and Eosin and examined under microscope. A few photomicrographs of representative types were also taken

STATISTICAL ANALYSIS-The result were expressed as mean ±S.D of six animals from each group for testing the difference among groups .The statistical analysis of variance were carried out by one way analysis of variance (ANOVA) with Fisher's post-hoc test. P values<0.05 were consider significant.

RESULTS-

Blood Serum activities of aminotransferases (ALT and AST) were significantly increased after paracetamol administration at all time intervals in comparison with control group ($p < 0.01$) (Table 1). A significant rise in blood serum MDA level was observed 6 h after paracetamol administration (27.10 ± 3.41 lmol/mg prot.) in comparison with control group (14.194 ± 3.64 lmol/mg prot.) ($p < 0.05$). After this period, MDA level showed a gradual increase and reached its highest value 48 h after treatment (29.28 ± 2.66lmol/mg prot.) (Table 1). MDA concentration was significantly increased in Paracetamole group in comparison with control group at all time intervals .Total SOD activity in the blood serum was significantly lower in P6 group (0.81 ± 0.13U/mg prot.) in comparison with control group (2.26 ± 0.17 U/mg prot.) ($p < 0.01$). Similar activities were measured 24h and 48h after paracetamol application (0.82 ± 0.18 and 29.28 ± 2.66 U/mg prot. in P24 and P48 group, respectively. The activity of SOD was decreased at all time intervals after paracetamol administration when compared to control group.

Table : Activity of liver enzymes, SOD and MDA in serum after PCM administration in different time interval:

SN.	Parameters	Control Group	Paracetamol treated group		
			6 hr.	24 hr.	48 hr.
1.	SGOT	37.33 ± 5.22	92.33 ± 5.29	93.88 ± 5.12	96.54 ± 4.26
2.	SGPT	29.21 ± 4.12	51.13 ± 5.22	52.99 ± 3.24	54.98 ± 3.98
3.	Alkaline phosphatase	29.10 ± 4.24	47.11 ± 4.43	47.17 ± 4.11	49.74 ± 3.12
4.	SOD	2.26 ± 0.17	0.81 ± 0.13	0.82 ± 0.18	0.86 ± 0.12
5.	LPO	14.194 ± 3.64	27.10 ± 3.41	28.78 ± 2.96	29.28 ± 2.66

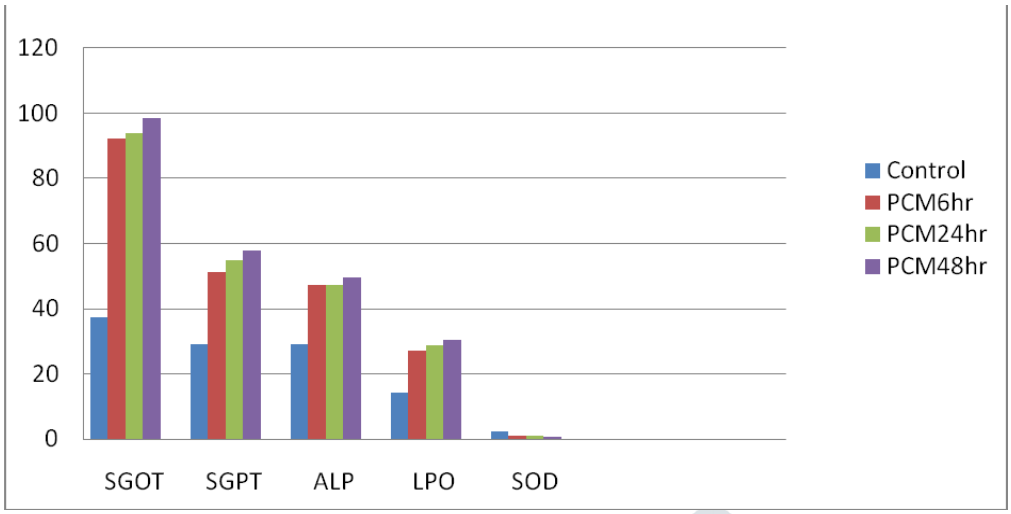


Fig. 1. Blood serum was collected 6, 24 and 48 h after administration of PCM. For testing the difference among groups, one-way ANOVA with Fisher's post-hoc test was used. *p < 0.05, **p < 0.01 in comparison with control group, ## p < 0.01 in comparison with Paracetamol induced group 6 h after administration.

HISTOPATHOLOGICAL STUDY OF PCM INTOXICATION IN DIFFERENT TIME INTERVALS-

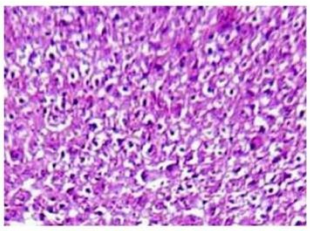
Histopathological study of liver tissue revealed that normal control group showed normal hepatic cells appear with intact cytoplasm and well defined nucleus and inflammatory cells were restricted. Whereas paracetamol induced group clear showed central vein dilated (CVD) along with hemorrhage and presence of inflammatory cells in hepatic cells, cytoplasmic membrane destruct and scattered lymphocyte and plasma cells was seen in around portal triad .

Fig. 1 : The representative microphotographs of H & E stained histological section of liver from rat showing normal architecture. (Control group)

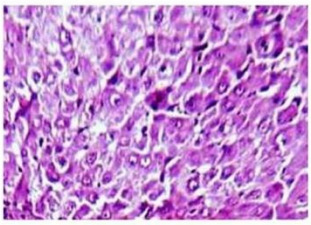
Fig. 2 : Microphotographs of H & E stained histological section of liver from rat treated with paracetamol (2g/kg *bw.*) showing centrilobular necrosis with hydropathic changes. (6hrs group)

Fig. 3 : Microphotographs of H & E stained histological section of liver from rat treated with paracetamol (2g/kg *bw.*) showing cloudy swelling of hepatocytes around central vein, hydropic degeneration . (24hrs group)

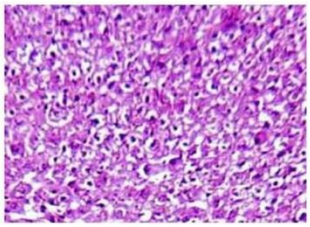
Fig. 4 : Microphotographs of H & E stained histological section of liver from rat treated with paracetamol (2 g/kg *bw.*) showing cloudy swelling of hepatocytes around central vein, hydropic degeneration and mild coagulative necrosis. (48hrs group)



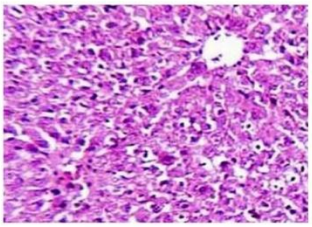
Control Group



PCM 6hrs Group



PCM 24hrs Group



PCM 48hrs Group

DISCUSSION-

Total number of 24 rats were included in the study and was divided into 2 groups, control as well as paracetamol treated group. Effect of paracetamol was observed at different time interval. On Paracetamol induced toxicity a significant rise in activity of aminotransferases, and alkaline phosphatase which indicates that this agent caused hepatocellular damage within first hours after treatment. We have shown that paracetamol led to increase in plasma MDA concentration 6 h after administration suggesting that lipid peroxidation plays an important role in the early phase of liver injury. According to other studies, the importance of lipid peroxidation in paracetamol-induced liver injury is controversial. There are numerous reports showing that paracetamol administration is closely followed by increase in lipid peroxidation products, leading to disturbance of cell membrane integrity. In addition, the importance of lipid peroxidation in acute paracetamol-induced liver injury was suggested by protective roles of a-lipoic acid and L-carnitine in hepatotoxic effects of this drug .. These various findings indicate that hepatotoxic effects of paracetamol are very complex and that various overlapping mechanisms, activated at different time points, are involved in mediating its hepatotoxicity. In our study a fall in total hepatic SOD activity was observed 6 h after paracetamol administration indicating that decrease in antioxidative capacity of hepatocytes may be a contributing factor to the oxidative liver injury. Decrease in SOD activity is in association with increase in reactive oxygen species production in early stage of paracetamol hepatotoxicity. The role of SOD depletion in the pathogenesis of paracetamol intoxication was, also, supported by numerous studies performed on different experimental models (Ajith et al., 2007; Dambach et al., 2006; Olaleye and Rocha, 2008). In vivo investigations showed that paracetamol in a dose of 250

mg/kg induced decrease in SOD activity 24 h after administration of this drug (Olaleye and Rocha, 2008). The amount of SOD reduction correlates with degree of hepatic injury (Dambach et al., 2006). Administration of various antioxidants alleviate paracetamol-induced reduction in SOD activity (Murugesh et al., 2005).

CONCLUSION

Based on results of our study, it can be concluded that paracetamol might disturb the balance between reactive oxygen species production and antioxidant protection in liver in the early phase of paracetamol hepatotoxicity. Lipid peroxidation was associated with liver injury and develop within first 6 h after paracetamol intoxication. These effects are maintained for the next 48 h. On the other hand, antioxidant capacity of hepatocytes is increased within 24 h. According to these findings, it can be estimated that antioxidant therapy should be administered as early as possible after paracetamol intoxication.

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