ORIGINAL ARTICLE

HEPATOPROTECTIVE ROLE OF ZINC SULPHATE IN ACETAMINOPHEN-INDUCED LIVER TOXICITY

Syed Hyder Raza Naqvi, Syed Qaiser Hussain Naqvi, Farkhunda Nadeem, Salman Ahmad Kazi, Navaid Kazi, Samina Shabir Rana, Sumayya Kazi

ABSTRACT

OBJECTIVE: To determine the role of zinc sulphate as a hepatoprotective agent in acetaminophen-induced histopathological changes in animal model.

DESIGN: Experimental observational study

SETTING: Department of Pharmacology & Pathology, ISRA University, Hyderabad and Department of Pathology, Peoples Medical College, Nawabshah.

DURATION: 1st December 2009 to 31st March 2010.

METHODOLOGY: Ninety healthy albino rats (weight 18-32 g) were divided into three main groups (n=30). Group A, which served as control, was maintained on 0.9% normal saline; Group B was given acetaminophen 250 mg/kg as a single dose; Group C was maintained on 1-5 mg/kg zinc sulphate for 1-7 days, before a single dose of acetaminophen 250 mg/kg. Biochemical studies were done 6 hours after acetaminophen administration. At the end of the treatment, all animals were weighed and sacrificed, the liver excised for gross and histopathological examination. Data were statistically evaluated using the Chi-square test.

RESULTS: The protective effect of zinc was demonstrated with the reduction in the levels of serum concentration of liver enzymes (aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and serum sorbitol dehydrogenase), with histopathological changes of centrilobular congestion, and hepatocellular degeneration and necrosis. Histopathological assessment showed typical pathological changes of centrizonal necrosis, steatosis, leukocyte infiltration, portal triaditis, and edema in those animals that received acetaminophen only. Pretreatment of the animals with zinc sulphate led to dose-dependent avoidance of these changes.

CONCLUSION: Zinc produces a hepatoprotective effect by preventing the ultrastructural injury of hepatic tissue and the disturbance of free amino acid metabolism caused by a toxic dose of acetaminophen.

KEY WORDS: Acetaminophen, liver toxicity, zinc, hepatoprotective effect

INTRODUCTION

Drug-induced liver injury is a potential complication of nearly every medication that is prescribed, because the liver is central to the metabolic disposition of virtually all drugs and foreign substances. Although drugs are usually metabolized without injury to the liver, many fatal and near-fatal drug reactions occur each year. A few compounds produce metabolites that cause liver injury in a uniform, dose-dependent fashion.[1-3]

Acetaminophen (*N*-acetyl-*p*-aminophenol, 4'-hydroxy-acetanilide, paracetamol) one of the most widely used analgesics, is toxic under conditions of overdose or in certain disease conditions. Although it is generally considered safe at therapeutic doses, in overdose, or in conjunction with liver disease and other disease conditions, acetaminophen displays toxicity, leading to morbidity as well as mortality.[4-5]

A great deal of work has gone into investigating the mechanisms by which acetaminophen is toxic[4,6-9] and is detoxified in mammalian systems. The major pathway for the removal of acetaminophen appears to be through glucuronidation and sulphation, which make it more water soluble and allow its removal from the liver and the blood via the urine.[10]

Zinc sulphate has been documented to offer protection by dose-dependently reducing alanine aminotransferase and malondialdehyde levels. The drug also partially prevented the depletion of hepatic glutathione. These effects were not as good as those of N-acetyl cysteine. However, the combination of zinc sulphate with N-acetyl cysteine produced even more protective effects. Furthermore, drug treatments did not affect serum acetaminophen levels. It is concluded that both drugs attenuate

Correspondence to:
Dr. Syed Hyder Raza Naqvi
Assistant Professor
Department of Pharmacology
Isra University & Hospital, Hala Road,
Hyderabad-Sindh, Pakistan.
E-mail: razahaiderdr@yahoo.com

acetaminophen-induced hepatic toxicity, and the action is likely to be mediated through replenishment of hepatic glutathione levels. The use of zinc sulphate alone or in combination with N-acetyl cysteine could be another alternative for the treatment of acetaminophen overdose in view of possible side effects produced by N-acetylcysteine.[11]

The aim of this study was to assess protection by zinc against acetaminophen-induced hepatotoxicity and to evaluate possible mechanisms of protection.

METHODS

This experimental observational study was conducted from 1 December 2009 to 31 March 2010 at the Department of Pharmacology and Pathology, ISRA University, Hyderabad, and the Department of Pathology, Peoples Medical College, Nawabshah. In this study, 90 healthy albino rats (weight 18-32 g) were divided in to three main groups (n=30). Group A served as control, Group B received acetaminophen, and Group C received acetaminophen and zinc sulphate.

EXPERIMENTAL DETAILS

Each group was administered the acetaminophen/zinc sulphate as follows: Group A: maintained on 0.9% normal saline, Group B: given acetaminophen 250 mg/kg as a single dose, and Group C: maintained on zinc sulphate for 7 days before a single dose of acetaminophen 250 mg/kg. Group C was further divided into three groups; C1 having zinc sulphate 1 mg/kg body weight , C2 maintained on zinc sulphate 3 mg/kg body weight, and C3 maintained on zinc sulphate 5 mg/Kg body weight.

SAMPLE COLLECTION

Blood samples: Blood samples were collected by cardiac puncture about 6 hours after acetaminophen administration, and used for biochemical studies. Biochemical assays for serum aspartate transaminase (AST) and alanine transaminase (ALT) were carried out with Randox kits (Randox Laboratories, UK) and based on the method of Reitman and Frankel.[12] Serum alkaline phosphatase was determined by the method of Armstrong and King.[13] Serum albumin was determined by reaction with BCG using Randox kits. The blood samples were allowed to clot at room temperature and the sera separated and stored at -20°C until required.
Tissue samples: At the end of respective period of time for the treatment, all animals of each group were weighed and sacrificed under ether anesthesia. A midline incision was made in the middle part of trunk and the liver was identified and removed. The gross appearance of the liver was observed for any change in color, consistency, shape, size, and contour with the help of a magnifying glass. After weighing and washing with normal saline, the liver was fixed in Bouin's fluid for 24 hours. The tissue was divided longitudinally in to two equal halves to observe the inner surface of both halves under dissecting microscope, then post fixed again in fresh Bouin's fluid for 24 hours. After processing in alcohol from 70%, 80%, and absolute for dehydration, the tissue was cleared in xylene and infiltrated and embedded in paraffin; 3- to 5-micron thick sections were cut and placed in a water bath at 40^0C-42^0C . The tissue sections were mounted on glass slides and stained with Periodic Acid Schiff iron hematoxylin. Detailed morphomatric observation of liver was performed with the help of an ocular micrometer scale and ocular reticule under the light microscope. Gomari rapid one-step trachoma was used to investigate the presence or absence of histopathological changes under 40X resolution; the changes were scored according to the zones of the hepatic lobule. Oil red-O stain was used for fat content of hepatocytes. Grading of fat content of liver cells was performed with ocular counting reticule under 40X objective in randomly selected areas of the hepatic lobule.

STATISTICAL ANALYSIS

The data were analyzed with the help of SPSS (Statistical Packages for Social Sciences) version 16.0 and expressed as mean ± SE. The statistical analysis for the comparison of means was evaluated using the student's t- test and the Mann-Whitney U test. Statistical significance was considered to be $p < 0.05$

RESULTS

The results revealed that the administration of acetaminophen to Group B animals caused a marked increase of serum transaminases and alkaline phosphatase concentration and a decrease in serum albumin levels. However, animals pretreated with zinc sulphate for 7 days produced a significant dose-dependent prevention of these biochemical changes. A significant improvement was noted in the biochemical parameters in group C animals when they were pretreated with zinc sulphate ($p< 0.05$ to $p < 0.001$). Furthermore, dose-related highly significant ($p < 0.05$ to $p < 0.001$) responses were observed when the dose of zinc sulphate was

increased in the subsequent groups (Table 1). Histological examination also showed typical pathological changes in Group B animals that received only acetaminophen (250 mg/kg). The important changes observed include central necrosis, steatosis, leukocytes infiltration, portal triaditis, and edema (Fig. 2).

Pretreated groups C1, C2, and C3 showed dose-dependent prevention of histological changes. Group C2 exhibited mild triaditis (Fig. 3) and minimal venous congestion. (Fig. 4) Histological analysis showed that there were no notable changes seen in Group C3 (Fig. 5).

DISCUSSION

Hepatotoxicity due to overdoses of acetaminophen (paracetamol) has become an important problem.[14,15] Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failure.[16,17] Acetaminophen is a commonly used analgesic and antipyretic agent, and its use is one of the most common causes of poisoning worldwide.[18] According to poison centers in the United States, acetaminophen poisoning was responsible for more than 70,000 visits to health care facilities and approximately 300 deaths in 2005.[19] Acetaminophen poisoning can be due to ingestion of a single overdose (usually as an attempt at self-harm) or ingestion of excessive repeated doses or too-frequent doses, with therapeutic intent. Repeated supratherapeutic ingestion is increasingly recognized as a significant clinical problem.[20,21] Acetaminophen is usually well tolerated in prescribed doses but overdose is the most common cause of drug-induced liver disease and acute liver failure worldwide, [22] which is one of the most painful experiences patients report. Reports of death from acute hepatotoxicity have been reported from as low as 2.5 grams over a 24-hour period. Damage to the liver is not due to the drug itself but to a toxic metabolite (N-acetyl-p-benzoquinone imine NAPQI, or NABQI) which is produced by cytochrome P450 enzymes in the liver.[23]

The frequent involvement of the liver in drug-induced toxicity depends on its anatomical location (the liver is the primary port of entry for ingested drugs) and its physiological and biochemical functions because of the abundance of metabolizing enzymes. The essentiality of zinc for humans was first documented in the 1960s. During the past 35 years, zinc deficiency in humans a result of nutritional factors and several disease states has been recognized. The protective effect of zinc reflected in the reduction of the increase of serum glutamic oxaloacetic transaminase (SGPT) and serum glutamic pyruvic transaminase (SGOT) activity was apparent after administration of acetaminophen.[24]

In our study, the pretreatment of albino rats with zinc sulphate prevented the escalation of serum liver enzymes such as AST, ALT, and alkaline phosphatase, and the decrease in serum albumin usually associated with acetaminophen hepatotoxicity. Similarly, the characteristic histological changes associated with acetaminophen toxicity such as centrizonal necrosis, steatosis, and sinusoidal enlargement were significantly prevented by zinc sulphate in a dose-dependent manner. Our observations are in agreement with an international study which indicated that zinc protects against acetaminophen- induced hepatotoxicity and that the observed protection is probably due to an induced biochemical change, but it is apparently not the result of any of the commonly invoked mechanisms.[25] Some researchers' use of therapeutic application of zinc sulphate as an antidote to acetaminophen overdose was examined in mice. Hepatotoxicity was induced by a single oral dose of acetaminophen (750 mg/kg). Various treatments (normal saline, 15 or 30 mg/kg zinc sulphate, 150 mg/kg N-acetyl cysteine, 15 mg/kg zinc sulphate + 150 mg/kg N-acetyl cysteine) were given 1 hour after acetaminophen overdose. However, the combination of zinc sulphate with N-acetyl cysteine produced even better protective effects. Furthermore, drug treatments did not affect serum acetaminophen levels. It is concluded that both drugs attenuate acetaminophen-induced hepatic toxicity, and the action is likely to be mediated through replenishment of hepatic glutathione levels. The use of zinc sulphate alone or in combination with N-acetyl cysteine could be another alternative for the treatment of acetaminophen overdose in view of possible side effects produced by N-acetyl cysteine.[11]

CONCLUSION

Zinc sulphate exerts significant dose-dependent protection of the liver against acetaminophen toxicity. The hepatoprotective effect of zinc was reflected by the significantly lower levels of SGOT, SGPT, serum alkaline phosphatase and serum albumin. Furthermore, the histological changes induced by acetaminophen overdose were also prevented by pre-administration of zinc sulphate.

REFERENCES

1. Mitchell JR, Jollow DJ, Potter WZ, Gillette JR,

Brodie BB. Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. J Pharmacol Exp Ther 1973;187:211-7.

2. Baerg RD, Kimberg DV. Centrilobular hepatic necrosis and acute renal failure in "solvent sniffers." Ann Intern Med 1970;73:713-20.

3. Klein AS, Hart J, Brems JJ, Goldstein L, Lewin K, Busuttil RW. Amanita poisoning: treatment and the role of liver transplantation. Am J Med 1989; 86:187-93.

4. Prescott LF. Paracetamol overdosage. Pharmacological considerations and clinical management.

5. Davidson DG, Eastham WN. Acute liver necrosis following overdose of paracetamol. Br Med J 1966;2(5512):497-9.

6. Howie D, Adriaenssens P, Prescott LF. Paracetamol metabolism following overdose: application of high performance liquid chromatography. J Pharm Pharmacol 1977;29:2357.

7. Ray SD, Kamendulis LM, Gurule MW, Yorkin RD, Corcoran GB. Ca^{2+} antagonists inhibit DNA fragmentation and toxic cell death induced by acetaminophen. FASEB J 1993;7:45363.

8. Ruepp SU, Tonge RP, Shaw J, Wallis N, Pognan F. Genomics and proteomics analysis of acetaminophen toxicity in mouse liver. Toxicol Sci 2002;65:13550.

9. Wu Y, Zhang X, Bardag-Gorce F, Robel RC, Aguilo J, Chen L, Zeng Y, Hwang K, French SW, Lu SC, Wan YJ. Retinoid X receptor alpha regulates glutathione homeostasis and xenobiotic detoxification processes in mouse liver. Mol Pharmacol 2004;65:5507.

10. Jollow DJ, Thorgeirsson SS, Potter WZ, Hashimoto M, Mitchell JR. Acetaminophen-induced hepatic necrosis. VI. Metabolic disposition of toxic and nontoxic doses of acetaminophen. Pharmacology 1974;12(4-5):25171.

11. Woo PC, Kaan SK, Cho CH. Evidence for potential application of zinc as an antidote to acetaminophen-induced hepatotoxicity. Eur J Pharm 1995;293:217-24.

12. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957;28:56-63.

13. King EJ, Armstrong AR. A convenient method for determining serum and bile phosphatase activity. Can Med Assoc J 1934;31(4):376-81.

14. Licht H, Seeff LB, Zimmerman HJ. Apparent potentiation of acetaminophen hepatotoxicity by alcohol. Ann Intern Med 1980;92:511.

15. Black M. Acetaminophen hepatotoxicity. Annu Rev Med 1984;35:577-93.

16. McNally, Peter F. GI/Liver Secrets: with STUDENT CONSULT Access. Saint Louis: C.V. Mosby 2006.

17. Ostapowicz G, Fontana RJ, Schiødt FV, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med 2002;137:94754.

18. Gunnell D, Murray V, Hawton K. Use of paracetamol (acetaminophen) for suicide and nonfatal poisoning: worldwide patterns of use and misuse. Suicide Life Threat Behav 2000;30:313-26.

19. Lai MW, Klein-Schwartz W, Rodgers GC, Abrams JY, Haber DA, Bronstein AC, Wruk KM. 2005 annual report of the American Association of Poison Control Centers' national poisoning and exposure database. Clin Toxicol (Phila) 2006; 44(6-7):803-932.

20. Daly FF, O'Malley GF, Heard K, Bogdan GM, Dart RC. Prospective evaluation of repeated supratherapeutic acetaminophen (paracetamol) ingestion. Ann Emerg Med 2004;44:393-8.

21. Schiodt FV, Rochling FA, Casey DL, Lee WM. Acetaminophen toxicity in an urban county hospital. N Engl J Med 1997;337:1112-7.

22. Keeffe EB, Friedman LM. Handbook of liver diseases. Edinburgh: Churchill Livingstone.2004;10423.

23. Wallace JL. Acetaminophen hepatotoxicity: NO to the rescue. Br J Pharmacol 2004;143:12.

24. Szymańska JA, Swietlicka EA, Piotrowski JK. Protective effect of zinc in the hepatotoxicity of bromobenzene and acetaminophen. Toxicology 1991;66:81-91.

25. Chengelis CP, Dodd DC, Means JR, Kotsonis FN. Protection by zinc against acetaminophen induced hepatotoxicity in mice. Fundamental and Applied Toxicology 1986;6:27884.

**TABLE 1: ACETAMINOPHEN-INDUCED BIOCHEMICAL CHANGES IN ANIMAL TISSUE (LIVER) WITH ZINC SULPHATE**

| GROUP	DRUG/TREATMENT	SGPT (ALT) U/L	SGOT (AST) U/L	ALKALINE PHOSPHATASE U/L	SERUM ALBUMIN G/L
A	5 mg/kg 0.9% N/saline	25.7 ± 4.7	55.6 ± 2.2	20.3 ± 1.7	42.8 ± 6.2
B	250 mg/kg acetaminophen	130.4 ± 3.6 ($p < 0.001$)	126 ± 3.1 ($p < 0.001$)	82.4 ± 3.9 ($p < 0.005$)	26.3 ± 2.1 ($p < 0.05$)
C1	(Pretreatment) 1 mg/kg zinc sulphate followed by 250 mg/kg acetaminophen	80.3 ± 3.1 ($p < 0.001$)	78.6 ± 2.7 ($p < 0.05$)	60.8 ± 2.9 ($p < 0.005$)	33.2 ± 3.5 ($p < 0.05$)
C2	(Pretreatment) 3 mg/kg zinc sulphate followed by 250 mg/kg acetaminophen	40.4 ± 2.7 ($p < 0.001$)	65.3 ± 2.2 ($p < 0.05$)	39.1 ± 2.1 ($p < 0.005$)	37.2 ± 2.9 ($p < 0.05$)
C3	(Pretreatment) 5 mg/kg zinc sulphate followed by 250 mg/kg acetaminophen	23.6 ± 3.3 ($p < 0.0001$)	58.4 ± 2.5 ($p < 0.05$)	22.5 ± 2.3 ($p < 0.001$)	40.2 ± 3.6 ($p < 0.05$)

ALT serum alanine transaminase
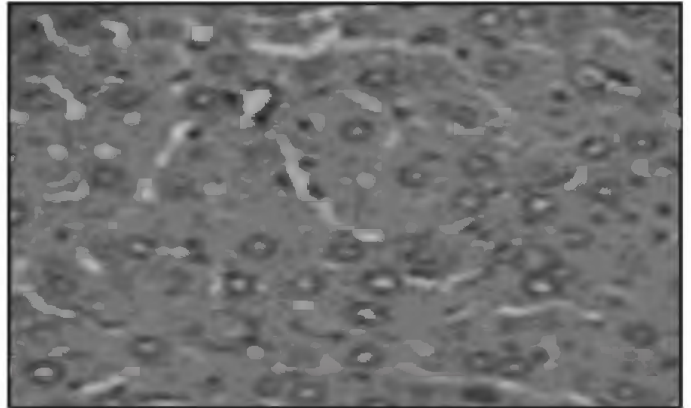AST serum aspartate transaminase
SGOT serum glutamic oxaloacetic transaminase
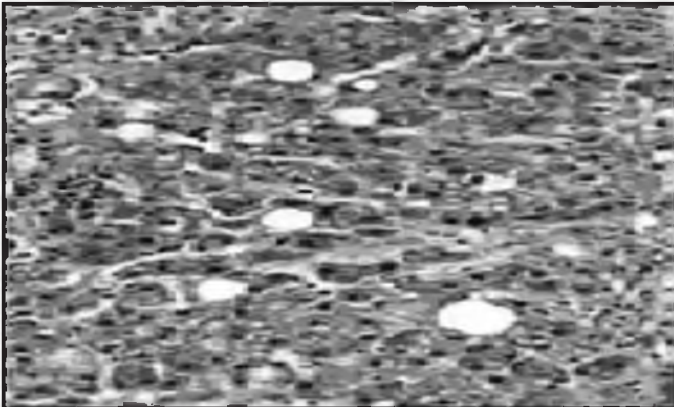SGPT serum glutamic pyruvic transaminase
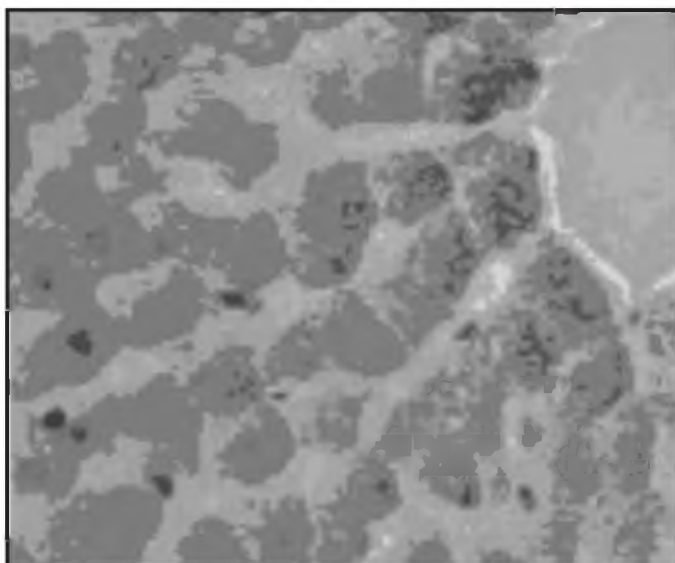
(FIGURE-I) NORMAL HEPATOCYTES ARRANGED IN TRABECULAR PATTERN
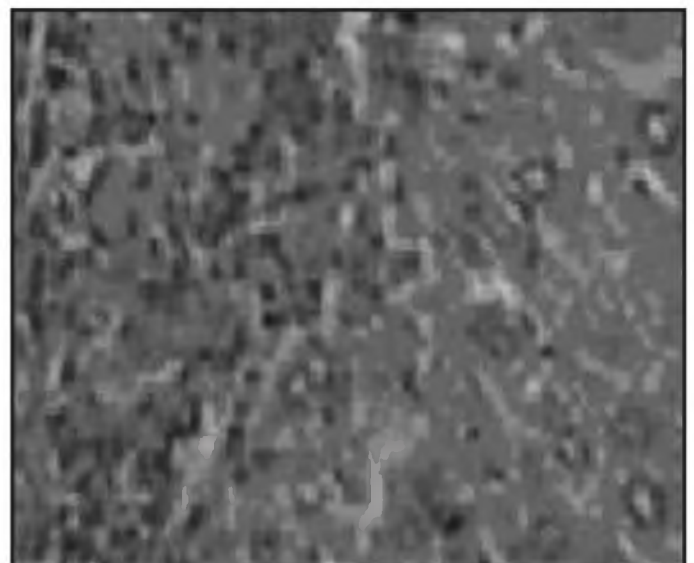
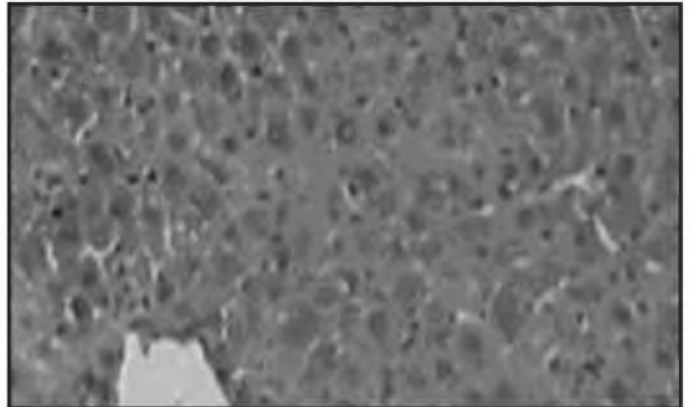**(FIGURE-II) EXTENSIVE HEPATOCYTE NECROSIS AND CENTRIZONAL NECROSIS IN GROUP B ALBINO RATS**
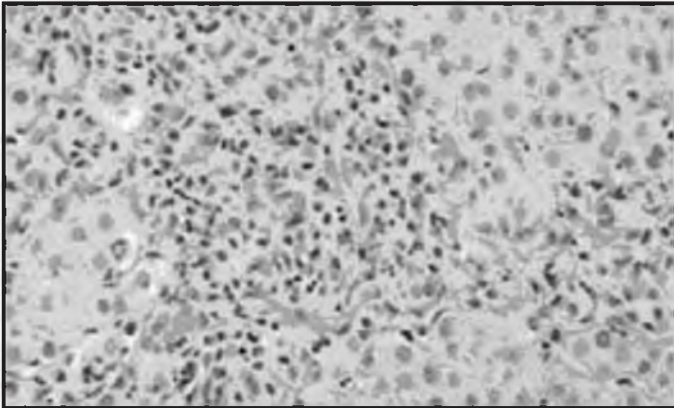
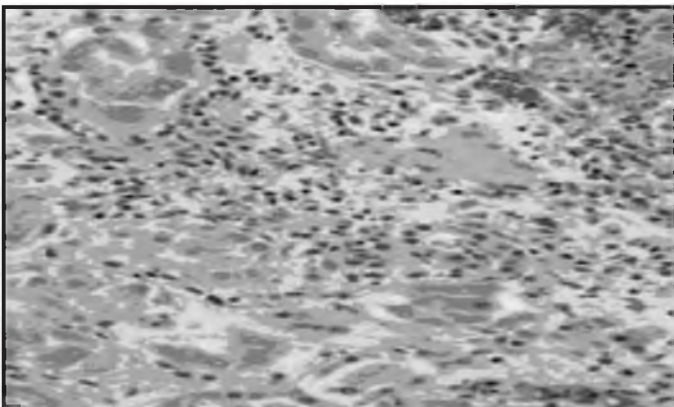**(FIGURE-III) MILD PORTAL TRIADITIS IN A SECTION FROM GROUP C1**

(FIGURE-VI) MILD VENOUS CONGESTION IN GROUP C2

(FIGURE-IV) MILD PORTAL TRIADITIS IN A SECTION FROM GROUP C2

(FIGURE-VII) NO REMARKABLE CHANGES IN GROUP C3

(FIGURE-V) MILD PORTAL TRIADITIS WITH INFLAMMATORY CHANGES IN A SECTION FROM GROUP C2