HEPATO PROTECTIVE EFFECT OF S-ADENOSYL-L-METHIONINE AGAINST ISONIAZID INDUCED HEPATITIS IN ANIMAL MODEL

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ABSTRACT

BACKGROUND

S-Adenosyl-L-Methionine (SAM) protects liver cells in viral hepatitis and paracetamol-induced hepatic damage, etc. But, there are scanty data regarding hepatoprotective effect of SAM in isoniazid-induced liver damage. With this background, the present study is undertaken to assess the activity of SAM in isoniazid-induced liver damage in animal model.

MATERIALS AND METHODS

Hepatic injury was induced in albino rats by administering isoniazid (INH) at a dose of 54 mg/kg orally once daily for 30 days and served as the animal model of hepatitis. S-Adenosyl-L-Methionine (SAM) at dose of 48 mg/kg once daily was administered one hour prior to administration of INH to other group of rats (SAM pretreated group) for same 30 days duration and act as the test group. Silymarin 100 mg/kg was used as the standard hepatoprotective drug in the study. The liver enzymes and bilirubin level in serum were measured in above treatment groups and were compared statistically. Histopathology of liver was also compared among different groups.

RESULTS

There was a significant lowering of liver enzymes in SAM pretreated group in comparison to the model group of INH-induced hepatitis. Histological examination also showed protective effect on liver architecture in rats pretreated with SAM followed by INH.

CONCLUSION

The results of the animal study clearly demonstrated the hepatoprotective effects of SAM against isoniazid-induced hepatitis.

KEYWORDS

S-Adenosyl-L-Methionine, Hepatitis, Isoniazid, Rat Model.

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BACKGROUND

Tuberculosis is one of the oldest disease known to affect humans. Over one third of world's population is infected with mycobacterium tuberculosis and more than 2 million people per year are dying of the disease.[1] A meta-analysis of studies involving several antitubercular drug regimens showed the incidence of hepatitis to be 2-6%, out of which hepatitis due to isoniazid alone attributes to 1.6%.[2] Isoniazid causes mild-to-moderate elevation of serum transaminase in approximately 0.5%-2% of patients.[3] The metabolite of isoniazid, i.e. hydrazine reacts with sulfhydryl group results in glutathione depletion within hepatocytes ultimately leading to cell death.[2] S-Adenosyl-L-Methionine (SAM) a metabolite of methionine is an important molecule required for many vital functions of cell as well as cellular survival. It is the principal biological methyl donor required for methylation of DNA, RNA, biological amines, phospholipids and other proteins.[4] In liver, SAM is a precursor of glutathione, a major endogenous antioxidant that protects cell against injury by scavenging free radicals involved in liver damage. Thus, SAM supplementation may protect the hepatocytes from any type of oxidative stress injury. Literature scan suggests SAM supplementation can afford protection to liver cells in liver disease, viral hepatitis and paracetamol-induced hepatic damage in both preclinical and clinical studies.[4] There are scanty data regarding hepatoprotective effect of SAM in isoniazid-induced liver damage.

Aims and Objectives

With the above background, the present study has been undertaken to assess the possible hepatoprotective activity of SAM in isoniazid-induced liver damage in animal model by evaluating the biochemical and histopathology parameters.

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MATERIALS AND METHODS

This was a randomised prospective experimental study conducted from May 2012 to August 2012 in the Department of Pharmacology, SCB Medical College, Cuttack. Fifty (50) healthy albino rats of either sex weighing 150-200 grams were selected and randomly divided into five (5) groups of ten (10) rats in each group. They were housed in departmental animal house fed with standard diet and allowed to drink water ad libitum. Drugs and chemicals were administered for 30 days period once daily orally through gavage to different group of rats as mentioned in the plan of the study. Blood samples were collected by retro-orbital puncture under light ether anaesthesia on the 31st day of drug administration. The animals were sacrificed with high dose ether anaesthesia and liver tissue was collected for histopathological examination. The plan of the study was approved by animal ethics committee of SCB Medical College, Cuttack, vide the IAEC (Institutional Animal Ethics Committee) meeting held on date - 3.5.2011.

Drugs and Chemicals

1. S-Adenosyl-L-Methionine (SAM)- Tablet Heptral of Abbott India Ltd. containing S-Adenosyl-L-Methionine (SAM) 200 mg was dissolved in 20 mL distilled water, so that 0.1 mL contains 1 mg of SAM. This pre-preparation was used at a dose of 50 mg/kg/once daily (OD) orally through gavage in the rats, which was nearly 2 times more than maximum human therapeutic dose (1600 mg/day) recommended for liver disorder.[5]
2. Isoniazid (INH)- Tablet Solonex DT of Macleods Pharmaceuticals containing 100 mg dispersible tablet of INH was dissolved in 10 mL of distilled water so that 0.1 mL contains 1 mg of INH and this preparation was used at a dose of 54 mg/kg/OD per orally through gavage in the rats.[6]
3. Silymarin- Syrup maryliv of Emcee Pharma containing 35 mg/5 mL (0.7 mg/0.1 mL) were used at a dose of 100 mg/kg/OD, per orally through gavage in the rats.[7]

The above S-adenosyl-L-methionine and isoniazid preparations were purchased from local market. Silymarin (Maryliv sample) was available in our department.

Plan of the Study

Group of Rats	Category	Drugs Administered
Group 1 Group 2	Normal rats (vehicle) (-ve control)	DW (distilled water) SAM + DW
Group 3 Group 4	Isoniazid treated rats (+ve control) (Test)	INH + DW SAM + INH (INH was administered 1 hr. after treatment with SAM)
Group 5	Silymarin treated rats (standard hepatoprotective drug)	Silymarin + INH (INH was administered 1 hr. after silymarin)

Parameters Studied

a) Assessment of Liver Function

Liver function was assessed with the help of an auto analyser by estimating-

1. Liver enzymes, i.e. AST (aspartate inotransferase), ALT (alanine aminotransferase).
2. ALP (alkaline phosphatase).
   Serum bilirubin, i.e. total bilirubin and direct bilirubin.

b) Histopathology Study- Histological examination of liver tissue section with Haematoxylin Eosin (HE) stain under electron microscope.

Statistical Analysis

All data were expressed as mean ± SEM, statistical difference between means was determined by ANOVA, followed by Turkey Kramer's post hoc test.

RESULTS

The details of biochemical parameters of Liver Function Test (LFT) in all the group of rats are depicted in table 1.

Rats treated with distilled water alone (Group 1) or SAM (Group 2) showed normal Liver Function Test (LFT) evidenced by normal range of liver enzymes (AST, ALT, ALP), total and direct bilirubin. Histological findings showed normal liver architecture with presence of intact hepatocytes and portal triad (Fig 1).

INH treated (Group 3) rats showed significant rise in all biochemical parameters of (LFT) when compared to normal rats treated with DW. Histopathology revealed hepatotoxicity as evidenced by distorted hepatic architecture, i.e. portal tract is expanded with interface hepatitis, but no fibrosis noted (Fig 2).

In group 4 rats (INH administered 1 hour after treatment with SAM), there was significant reductions in AST, ALT, ALP and total and direct bilirubin in comparison to group 3 (INH) treated rats. Histopathologically, there was an almost normal architecture characterised by normal structure of intralobular vein. No pycnosis in the nucleus, but there was slight damaged wall of the intralobular vein (Fig 3).

In group 5 rats (INH administered 1 hour after treatment with silymarin), there was also significant reduction in AST, ALT, ALP, total and direct bilirubin in comparison to group 3 (INH) treated rats.

Groups n=10 Rats in Each Group	Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)
Group 1	DW	53.63 ± 3.06	30.23 ± 2.21	200.01 ± 3.06	0.61 ± 0.04	0.32 ± 0.03
Group 2	SAM + DW	58.75 ± /-3.45^{c}	38.65 ± 2.08^{c}	219.07 ± 7.43^{c}	0.65 ± 0.12^{c}	0.38 ± 0.03^{c}
Group 3	INH	299.01 ± 3.37^{b}	82.01 ± 6.04^{b}	325.6 ± 7.9^{b}	0.85 ± 0.03^{a}	0.46 ± 0.03^{a}
Group 4	SAM + INH	143.32 ± 3.10^{d}***	75.31 ± 0.02^{e}**	213.02 ± 10.11^{e}***	0.65 ± 0.02^{e}*	0.35 ± 0.01^{e}*
Group 5	Silymarin + INH	60.24 ± 2.53***	61.21 ± 2.5***	203.02 ± 0.35***	0.61 ± 0.02**	0.33 ± 0.01**
F value		253.37	160.27	165.8	65.8	22.63

Table 1. Liver Enzymes and Bilirubin Level in Rats Treated with SAM, INH and Silymarin (for Analysis of INH-Induced Hepatotoxicity)

N=10, data were expressed as mean+/-SE, groups were compared by one-way ANOVA, intergroup comparison by post hoc analysis.

1. ^{c}p >0.05, ^{a}p <0.01, ^{b}p <0.001 when compared with DW.
2. *p <0.05, **p <0.01, **p <0.001 when group 4 and group 5 are compared with INH treated group (group 3).
3. ^{d}p <0.05, ^{e}p >0.05 when group 4 compared with group 5.

Figure 1. Normal Liver Architecture of Rat Treated with DW

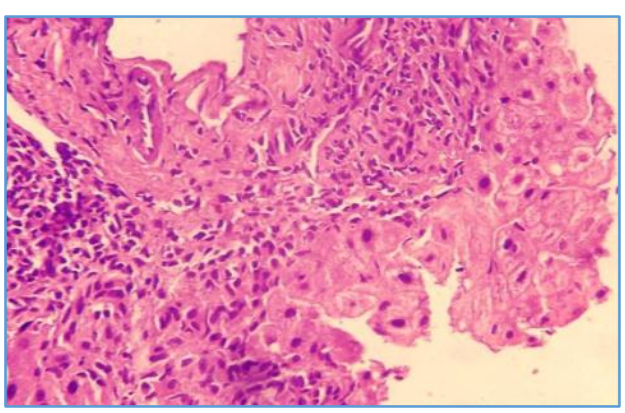

Figure 2. INH-Treated Rats Showing Interface Hepatitis

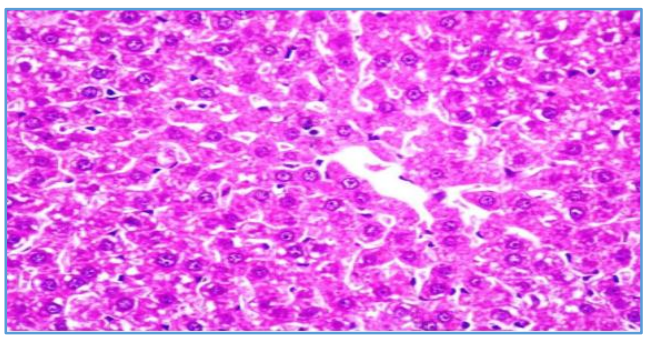

Figure 3. SAM Pretreated Rats showing Protection Effect on Liver Architecture

DISCUSSION

In the present study, we have tried to evaluate the effect of S-Adenosyl-L-Methionine (SAM) in INH-induced hepatotoxicity by evaluating both the biochemical and histopathological parameters. The liver enzymes, the bilirubin level (biochemical parameters) and the hepatic histopathology was found out in normal rats treated with Distilled Water (DW) and were compared with that of SAM, INH and SAM followed by INH treated rats. Rats treated with SAM alone showed normal liver function test (AST, ALT, ALP and bilirubin level) and histological findings (intact hepatocytes and portal triad) like that of DW treated rats.

INH treated (group 3) rats showed significant rise in all the biochemical parameters, i.e. levels of AST, ALT, ALP and bilirubin level indicating hepatocellular damage. Physiologically, ALP is excreted into bile, but during liver injury, it is unable to be excreted through bile and its level increase in serum.[8] Increased level of ALP in systemic circulation maybe associated with hepatocyte damage. Increased in serum bilirubin (jaundice) observed in INH treated rats is suggestive of impaired liver function due to decrease clearance.[9] The above findings due to INH treatment may be attributed to formation of hydrazine (direct process) and acetyl hydrazine (indirect process), both metabolic products are hepatotoxic.[10] Reports in rats also suggest that the hydrazine metabolite of INH and its subsequent effect on CYP2E1 induction is involved in INH-induced hepatotoxicity.[10] In addition to the above-mentioned mechanism, oxidative stress induced by INH is also another important way for INH-induced hepatitis.[11]

In the present study, SAM alone did not produce any change in biochemical and histopathological parameters in

rats (Table 1). But, SAM followed by INH treated (Group 4) rats exhibited beneficial effects of SAM as evidenced by normalisation of all the biochemical parameters (post hoc analysis demonstrated that the estimated values of liver enzymes, i.e. AST, ALT, ALP, total and direct bilirubin of group 4 rats are significantly lower than INH-treated (group 3) rats ($P < 0.01$-0.001 for liver enzymes and < 0.05 for bilirubin, Table 1). Rather the levels of biochemical parameters of SAM pretreated group are comparable to silymarin pretreated group ($P > 0.05$ for all the parameters except AST when group 5 rats are compared with group 4, table 1). So, in intact animals prior administration of SAM may provide protection against INH-induced liver injury through various mechanisms. One of them maybe restoration of hepatic concentration of glutathione by SAM leading to desirable scavenging action of INH generated free radicals.[12,13]

In our study, the hepatoprotective effect of SAM was further confirmed by histological findings, i.e. almost normal liver architecture and absence of infiltration of mononuclear cells in SAM followed by INH-treated rats (Figure 3) in comparison to expanded portal tract with interface hepatitis with infiltration of mononuclear cells noted in INH-treated rats (Figure 2). This protective effect of pretreatment with SAM maybe due to elevation of glutathione concentration, which protects the hepatocytes from oxidative stress. Literature scan further revealed that increase glutathione concentration also protect hepatocytes from tumour necrosis factor-alpha toxicity such as necrosis.[14] Absence of inflammatory response with SAM maybe attributable to decrease TNF-alpha concentration as reported by Chawla et al, 1998.[15]

CONCLUSION

The present study showed that S-adenosyl-L-methionine possess hepatoprotective effect against INH-induced hepatotoxicity as evidenced by normalisation of liver enzymes and protection of liver architecture in S-adenosyl-L-methionine pretreated rats.

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