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HEPATOPROTECTIVE EFFECT OF GARLIC (ALLIUM SATIVUM) AND MILK THISTLE (*SILYMARIN)* IN ISONIAZID INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Objective: Assessment and comparison of hepatoprotective role of garlic and silymarin in anti-tuberculosis drug (Isoniazid) induced hepatotoxicity.

Materials and Methods: Healthy rats weighing 150 – 200g were selected for the study. Rats were divided into four groups, i.e. group A (Control), group B (INH treated group), group C and D serve as experimental groups. The group A received only the normal diet, Group B along with normal diet received the isoniazid (50 mg/kg/day), Group C along with normal diet received the isoniazid (50 mg/kg/day) and Silymarin (200 mg/kg/day), and Group D along with normal diet received the isoniazid (50 mg/kg/day) and garlic (0.25 g/kg/day).

Results: On completion of 28 days of treatment blood samples were collected for the assessment of liver function. In group B there was abnormal rise in the levels of biochemical markers (ALT, AST, ALP and Total Bilirubin). In group C the biochemical markers were near normal levels and in group D the levels of biochemical markers were within normal limits.

Conclusion: Both the garlic and silymarin have shown the hepatoprotective effect against the Anti-tuberculosis drug (isoniazid) induced hepatotoxicity in experimental animal model.

Key words: Antituberculosis therapy (ATT), Isoniazid (INH), National Institute of Health (NIH)

INTRODUCTION

The historic evidence about the prevalence of tuberculosis dates back to around 8000 B.C.[1] About three million deaths are caused by *Mycobacterium Tuberculosis* worldwide and there is an increase in number of new cases as well.[1] Countries with a very high number of cases of Tuberculosis are considered as high burden countries (Bangladesh, China, India, Indonesia, and Pakistan).[2] It was estimated that some 7–8 million new cases and 2 – 3 million deaths occur annually in the world.[4-5] The adverse effects especially drug induced hepatotoxicity is one of the main reasons of noncompliance to the treatment regimens for *tuberculosis* (TB).[3] The use of drug for the treatment of tuberculosis results in decrease in the glutathione, that results in free radical injury because glutathione prevents free radical injury.[2,3] Moderate GSH depletion accompanied the injury and was associated with lipid peroxidation. There is a greater risk of hepatotoxicity when there is co-administration of the INH and Rifampicin combination.[6] The antioxidant activity of garlic results in inhibition of lipid peroxidation.[6] The main major active constituent of Silymarin shows hepatoprotective activities.[7]

Keeping all these activities in mind the present experiment was designed to study the preventive role of garlic and silymarin against hepatotoxic effects of INH.

MATERIALS AND METHODS

Setting:

Study was conducted on 40 healthy male rats which were kept in animal house of National Institute of Health, Islamabad, Pakistan.

Design:

It is a randomised controlled experimental study on male rats.

Animals used in the study:

In the study 40 rats weighed 150 – 200 g were selected. Rats were divided in four groups (10 in each group). Each group was kept in separate cage in the same room and under similar conditions (Temperature 22 ± 2°C and 12 hours light / dark cycle) in animal house at NIH. Initially all groups were fed on standard diet (starch, vitamins, minerals and fats) and water for 01 week for acclimatisation before starting the experiment.

Group A: Control fed on normal diet and received no drug (n = 10).

Group B: Was administrated Isoniazid orally (n= 10).

Group C: Was administrated Isoniazid (50 mg/kg body weight / day) and Silymarin (200 mg/kg body weight / day) simultaneously through oral route (n = 10).[7]
Group D: It was administrated Isoniazid (50 mg/kg body weight / day) and Garlic (0.25 g/kg body weight / day) simultaneously through oral route (n = 10).[14]

Drug, dosage, rout of administration and duration:
The following antituberculosis drug was used in this study:
Isoniazid (INH): 50 mg/kg body weight through oral rout of administration once daily for 28 days.[32]

Experimental plant material:
Garlic and Milk thistle (Silymarin) used in this study:
Garlic was collected from the local vegetable market (sabzimandi) Islamabad. Freshly collected garlic was washed in tap water. Silymarin was obtained from commercially available suspensions from standard medical store. Garlic 0.25 g/kg body weight / day administrated through oral rout, once daily for 28 days.[32] Silymarin 200 mg/kg body weight / day administrated through oral route, once daily for the 28 days.[33]

Experimental Procedure
Phase 01
In the study 40 male rats weighted 150 – 200 gm were selected. The rats were divided into four groups (10 in each group). Each group was kept in separate cage in the same room and under similar physiological conditions in animal house in NIH.

Initially all groups were fed on standard diet (starch, vitamins, minerals fats) and water for 01 week for acclimatisation before starting the experiment.

Phase 02
Baseline blood sample in all the rats in groups were taken after 01 week for acclimatisation.

Two ml of blood was drawn with the 3 ml syringe from tail vein. Blood was transferred to labelled centrifuged tubes and allowed to clot at room temperature for 01 hour. Samples were centrifuged for 10 minutes at 300 rev / min. Serum was separated and stored at –20°C and analysed for liver enzymes, using semi-automated chemistry analyzer.

Phase 03
Group A: Rats considered as control, receiving no medicine.
Group B: Rats were administrated 50 mg/kg of INH once daily for 28 days.
Group C: Rats were administrated 50 mg/kg/day of INH and 200 mg/kg/day Silymarin was administered orally once daily for 28 days.
Group D: Rats were administrated 50 mg/kg/day of INH and 0.25 g/kg garlic orally once daily for 28 days.

Phase 04
Blood samples from all the Rats (in all four groups) were taken after 28 days of therapy. Two ml of blood is drawn with the 3 ml syringe by cardiac puncture. Blood was transferred to labelled centrifuge tubes and allowed to clot at room temperature for 01 hour. Then samples were centrifuged for 10 minutes at 300 rev / min. Serum was separated and stored at –20°C and analysis for the liver enzymes, were done through semi-automated chemistry analyser.

Biochemical Analysis
Serum ALT, AST, ALP and total Bilirubin levels were estimated by commercially available kits (Randox of UK). Serum ALT, AST, ALP was estimated by IFCC method.[26-30] Total bilirubin was estimated according to calorimetric method.[34]

Statistical Analysis
The data was entered and analysed using Statistical Package for Social Sciences (SPSS 17.0). All data was shown as mean ± S.E.M. One way ANOVA was applied to observe group mean differences. Post Hoc Tukey test was applied to observe mean differences among the groups. A p-value of <0.05 was considered as statistically significant.

RESULTS
In all the four groups there was no mortality during the course of the study. In control group A receiving only the normal standard diet and water. The levels of liver enzymes and total bilirubin remained within normal limits. In case of group B which received orally 50 mg/kg of INH daily along with the standard diet and water for 28 days, there were abnormally elevated levels of the liver enzymes and total bilirubin as compared to the Control group A. In the group C treated orally with 50 mg/kg of INH daily for 28 days and 200 mg/kg/day Silymarin was administered orally half an hour before the INH dose. The levels of liver enzymes and total bilirubin were close to normal limits. In group D orally with 50 mg/kg/day of INH daily each for 28 days and 25 g/kg per day of garlic was administered orally half an hour before the INH dose, the levels of liver enzymes and total bilirubin were within normal limits.

DISCUSSION
In order to investigate the INH induced hepatotoxicity many animals (rabbits, rats etc) have been

Biochemical Markers / Groups	*Group A*	*Group B*	*Group C*	*Group D*
ALT U/L	30.5 ± 0.838	130.9 ± 1.9606[a]	36.4 ± 0.6261[b]	28.1 ± 0.5145[b]
AST U/L	101.4 ± 1.8764	272.7 ± 4.4709[a]	127.4 ± 1.5633[b]	98.5 ± 1.2268[b]
ALP U/L	123.6 ± 1.31	385.6 ± 2.5542[a]	143.8 ± 1.1827[b]	118.4 ± 1.2268[b]
Total Bilirubin levels (mg/dl)	0.477 ± 0.0496	2.4673 ± 0.1115[a]	0.629 ± 0.0387[b]	1.328 ± 0.0329[b]

a $p < 0.05$ b $p < 0.01$

used however in the present study rats were used to assess the role of garlic and silymarin in the prevention of INH induced hepatotoxicity in rats.[8-12] Among the first line antituberculous drugs the most important drug is the INH. In 20% of the patients hepatotoxicity is caused by the INH.[18] The hepatotoxic role of INH has been studied and well documented in previous studies.[9-13] The mechanism involved in the hepatic injury caused by the INH is the oxidative stress. The reduced form of the glutathione has a major role in the removal of the naturally occurring free radicals.[19]

In this study the drug (INH) induced hepatotoxic effect has been observed in the group B rats receiving INH. The abnormal rise in the levels of serum ALT, AST, ALP and total bilirubin has been observed in the group B (receiving INH 50 mg/kg per day each) as compared to the group A receiving only the standard diet. The elevated levels of these biochemical markers in the serum are the indicators of hepatotoxicity. On the other hand the hepatoprotective role of garlic and milk thistle has been observed in the group C (receiving INH and milk thistle 200 mg/kg/day) and D (receiving INH 50 mg/kg per day each and 0.25 g/kg per day of garlic) respectively. The levels of liver enzymes ALT, AST, ALP and total bilirubin showed no abnormal rise as compared to group B and remained close to normal levels.

The progression of steatosis to steatohepatitis and cirrhosis results due to the fact that accumulated lipid in the liver induce the inflammation, apoptosis and fibrosis [20]. In the liver reactive oxygen species (ROS) are frequently produced as liver is the major site for the normal metabolism and the drug metabolism. To tackle this problem of the free radicals liver has a unique system comprising of the glutathione mechanism. This defensive system becomes impaired if there is depletion of the reduced form of the glutathione of 20% or more.[21]

The previous studies showed that several plants have hepatoprotective effects against the hepatotoxic effects of xenobiotics including those used in tuberculosis treatment.[10,14-17] The biochemical constituents of garlic bulb are in following proportions containing approximately 65% water, 28% carbohydrates (mainly fructans), 2.3% organosulfur compounds, 2% protein (mainly alliinase), 1.2% free amino acids (mainly arginine)and 1.5% fiber. A large amount of γ-glutamylcysteine is present in the garlic bulb. There are a number of diseases which are caused by the oxidative stress, are prevented by the antioxidants and detoxifying properties in the garlic.[22] The phase I reactions are inhibition and induction of the phase II reaction by the garlic.[23] Consumption of garlic enhances the intracellular contents of glutathione in all cells including those in normal liver and mammary tissue.[24]

The garlic also has an anti inflammatory effect as it decreases pro inflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-8, T cell interferon gamma, IL-2 and enhances the production and function of anti-inflammatory monocyte IL-10.[36]

It shows that several enzymes of the cytochrome P450 are inhibited thus decreasing the production of toxic metabolic intermediates.[25-26] In addition to its hepatoprotective actions, silymarin is shown to be effective in other organs including lung and brain; and to inhibit tumour growth and promotion in several types of cancer.[27-28] Amongst the flavonoids, which have proven antioxidative, antiviral or anticarcinogenic properties like glycyrrhizin, phyllanthin, silybin, picroside and baicalein, can serve as primary compounds for further development as hepatoprotective drugs.[35]

Previous studies have shown that the safety profile of the silymarin is very good.[29] The results are consistent with the previous studies which clearly indicate the hepatoprotective role of milk thistle and garlic against the antituberculosis drug induced hepatotoxicity.[30-33]

In this study it has been shown that INH induced hepatotoxicity was prevented by the simultaneous use of garlic and milk thistle along with INH. Thus to prevent the INH induced liver injury can be prevented by using garlic simultaneously along with the INH. Further trials are necessary regarding dose, rout of administration, formulation and combination therapy of garlic and milk thistle to assess their full benefits.

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