Original Article

Antituberculous Treatment Induced ALT & ALP Derangements and The Role of Onion Extract in Male Albino Rats

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ABSTRACT

Objective: Study was designed to investigate the biochemical derangements induced by antituberculous drugs and role of onion extract in male albino rats. **Period;** March to December 2008 **Setting**; Study was conducted on non-tuberculous male albino rats, which were kept in animal house of University of Health Sciences, Lahore.

Materials and Methods: 48 male albino rats, obtained from NIH Islamabad weighing 200-250 gms were divided into four groups, each group consisting of 12 rats.

Group A; received normal diet and fresh water orally. Group B; received normal diet, fresh water and antituberculous drugs orally. Group C; Received 100mg/kg body weight of onion extract and antituberculous drugs orally and Group D; Received 200mg/kg body weight of onion extracts and antituberculous drugs orally, Rats were treated with this regimen for 45 days. After the last administration, blood samples were collected. Serum samples were separated into sterile appendorf tubes and stored at -20 C until used for estimation. Following methods were followed for biochemical investigations.

Estimation of Serum Alanineaminotransferase (ALT) and estimation of serum Alkaline Phosphatase (ALP).

Results; In this biochemical study; There was a significant elevation in the levels of serum diagnostic marker enzymes (ALT, ALP) in group B antituberculosis drugs administered rats as compared to that of non-antituberculosis drugs administered in group A. Co-administration of onion extract and antituberculous drugs, decreased enzyme levels were noticed in a dose dependent manner in group C and group D rat's serum samples.

Conclusion; Antituberculous drugs causes significant elevations in the level of serum diagnostic marker enzymes (ALT and ALP) and onion extract lowers the enzyme levels indicating its cytoprotective effect.

Key Words: Marker Enzymes, Antituberculous

INTRODUCTION

Liver plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, and detoxification. It produces bile, an alkaline compound which aids in digestion, via the emulsification of lipids. It also performs and regulates a wide variety of high-volume biochemical reactions requiring very specialized tissues.[1]

Currently, there is no artificial organ or device capable of emulating all the functions of the liver. Some functions can be emulated by liver dialysis, an experimental treatment for liver failure. Many diseases of the liver are accompanied by jaundice caused by increased levels of bilirubin in the system. The bilirubin results from the breakdown of the haemoglobin of dead red blood cells; normally, the liver removes bilirubin from the blood and excretes it through bile.[2]

Antituberculous therapy is associated with significant hepatotoxicity. This often results in discontinuation of the most effective first line drugs such as, ISONIAZID, RIFAMPICIN, and PYRAZINAMIDE. Tuberculosis is highly prevalent in developing countries. It is re-emerging in developed countries as a result of the pandemic of HIV infection, increase in the organ transplant population and transglobal migration.[3]

Hepatotoxicity manifests as clinical jaundice and multilobular necrosis. Isoniazid induced hepatic dysfunction is due to chemically reactive metabolite

Acetyl hydrazine. It can be detected in 10% of cases. Incidence is more in elderly and alcoholics. Clinical hepatitis requires immediate withdrawal of drug.[4]

Drug-induced severe hepatotoxicity is a major health problem with increasingly significant challenges to modern hepatology. Drug-induced liver injury has been a long-standing concern in the treatment of tuberculosis infection. Biochemical diagnosis of drug-induced liver injury depends on non-specific elevation in liver tests , in particular, increases in serum aminotransferases activity and bilirubin levels.[5]

Antituberculosis drugs produce progressive enhancement of toxicity over 15-90 days. They cause membrane damage resulting in leakage of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and serum billirubin . [6]

Administration of Antituberculous drugs to Wistar rats results in cytolytic liver injury. It has been established in experimental animals that antituberculous drugs administered in toxic doses affect the liver, its membranes and cellular organelles. Consumption of a flavonoid rich diet might decrease the risk of degenerative changes in certain diseases.[7]

MATERIALS AND METHODS

The experimental study involves group of animals (albino rats) treated with antituberculous drugs and a combination of antituberculous drugs and aqueous onion extract in variable doses. The changes in liver functions were studied in these groups. **Study design;** It is randomized control experimental study on male albino rats. **Study Setting;** Study was conducted on 48 non-tuberculous, male albino rats and they were kept in animal house of University of Health and Sciences, Lahore.

Duration of Study; March to December 2008.**Sample Size;** A total of 48 male albino rats, obtained from NIH Islamabad weighing 200-250 gms were divided into four groups, A,B,C and D each group consisting of 12 rats.

Preparation of Experimental Animals; Adult male albino rats weighing 200-250g were included in this study. They were divided into four groups, each having 12 rats. Before the commencement of the experiment, all animals were kept for one week under the same laboratory conditions, at temperature of (22 ± 2 degree Celsius) relative humidity (70 ± 4%), and 12 hour light/day cycle. They received a nutritionally standard diet and tap water.

Preparation of onion extract: Aqueous onion extract was prepared from fresh onion. The onions were peeled on crushed ice and 50g of onion was homogenized in 75ml of cold 0.9% NaCl in the presence of some crushed ice. The homogenization was carried out in a blender at high speed using two minutes bursts for a total of twelve minutes. The homogenized mixture was filtered three times through cheese cloth. The filtrate was centrifuged at 2000 ×g for 10min and clear supernatant was made up to 100ml with normal saline. This aqueous extract of onion was stored at -20°C until use. It was standardized from PCSIR laboratories, Lahore,Pakistan.[8]

DRUGS AND DOSAGES:

Following antituberculous drugs dosage were used [9]

1. ISONIAZID → 7.5mg/kg body weight (O.D)
2. RIFAMPICIN → 10mg/kg body weight (O.D)
3. PYRAZINAMIDE →35mg/kg body weight(O.D)

Experimental plant material was

ONION EXTRACT → 100mg/kg body weight (O.D)
200mg/kg body weight (O.D) [10]

All these drugs were administered orally daily, mixed with distilled water.

EXPERIMENTAL PROCEDURE:

After habituation period the rats were divided into four groups randomly, each of 12 rats.

The details of the groups are mentioned below:

Group A:- (Normal control group)
Received normal diet and fresh water orally.

Group B:- (ATT control group)
Received Antituberculous drugs orally in doses mentioned above, Once daily

Group C: - (Experimental group 1)
Received 100mg/kg body weight of onion extract + Antituberculous drugs, orally, once daily.

Group D: - (Experimental group 2)
Received 200mg/kg body weight of onion extract + Antituberculous drugs orally, once daily.

Rats were treated with this regimen for 45 days.

COLLECTION OF SAMPLES: A day after the last administration, the animals were anaesthetized using chloroform vapours and their blood samples were collected by performing cardiac puncture in sterile vacutainers with gel. Serum samples were separated from the clot after centrifugation at 3000 rev/min for 5 min, using bench top centrifuge. Serum samples were separated into sterile appendorf tubes and stored at

-20°C until used for estimation. All analyses were completed within 24hrs of sample collection.[11]

LABORATORY INVESTIGATIONS:-

Following liver function tests were carried out;

1. Serum Alanin Aminotransferase (ALT)
2. Serum Alkaline Phosphatase (ALP)

Followings are the methods which were followed for biochemical investigations; **Estimation of Serum Alanine aminotransterase (ALT):**

Reagent

R1: ALT Substrate. TRIS buffer 140 mmol/L pH 7.8, L-alanine 700
mmol/L, lactate dehydrogenase > 2300 U/L.

R2: ALT coenzyme. NADH 1 mmol/L, 2-oxoglutarate 85 mmol/L

Reagents were prepared by adding 4ml of R1 to 1ml of R2. They were stored at 2-8 ^{o}C. **Assay Procedure:** Plasma ALT was determined by using ALT kit, manufactured by DiaSys Diagnostic Systems, Germany, with Lot No. 505 on Metrolab 2300 Chemistry Analyzer by IFCC method. Reagent was placed into the reagent disc. Controls were run followed by test samples, 0.4 ml of non -hemolyzed sample was placed into the sample cup and analysis was done. The change in the absorbance was measured at 340 nm by using kinetic mode with a factor -2300. Control materials used were, Assayed bovine sera level 2 (Normal) Lot No: 355SN and Assayed bovine sera level 3 (Abnormal) Lot No: 280SE/1 of Randox (UK). CV of the method was 0.64- 0.74 percent.[12]

Estimation of Serum Alkaline Phosphatase (ALP):

Reagent

R1: ALP Substrate, pH 9.8, diethanolamine 1.25 mmol/L, magnesium chloride 0.625 mmol/L.

R2: AST coenzyme. NADH 1.3 mmol/L, 2-oxoglutarate 65 mmol/L. Reagents were prepared by adding 4ml of R1 to 1ml of R2. They were stored at 2-8 ^{o}C.

Assay Procedure: Plasma ALP was determined by using ALP kit, manufactured by DiaSys Diagnostic System, Germany,with Lot No. 505 on Metrolab 2300 Chemistry Analyzer by IFCC method. Reagent was placed into the reagent disc. Controls were run followed by test samples, 0.4 ml of non -hemolyzed sample was placed into the sample cup, and analysis was done. The change in the absorbance was measured at 340 nm by using kinetic mode with a factor -2300. Control materials used were, Assayed bovine sera level 2 (Normal) Lot No: 355SN and Assayed bovine sera level 3 (Abnormal) [13]

STATISTICAL ANALYSIS: The data was entered and analyzed using SPSS 17.0 (Statistical Package for Social Sciences). Mean ± S.E.M is given for quantitative variables. Frequencies, percentages and graphs are given for qualitative variables. One way ANOVA was applied to observe group mean differences. A p-value of <0.05 was considered as statistically significant.

RESULTS

In this study, hepatotoxicity was induced by antituberculosis drugs, e.g Isoniazid, Rifampicin and Pyrazinamide, for evaluation of the protective effect of aqueous extract of Allium cepa linn (onion) in a dose dependent manner, by giving dose of onion extract 100mg/kg to group C rats and 200mg/kg/body weight to group D rats, along with antituberculosis medicines. After treating the rats for 45 days the biochemical analysis of Liver function tests including Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) were carried out.

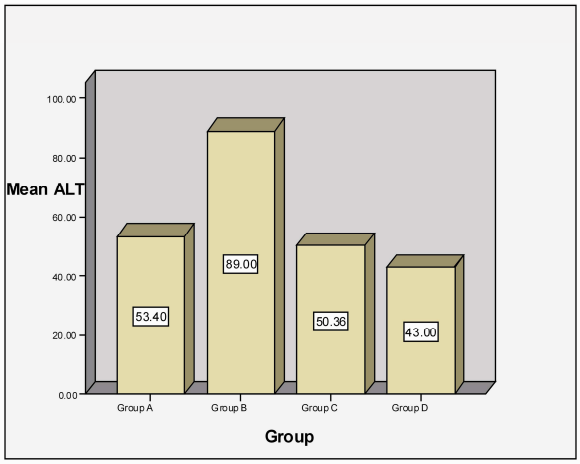
There was a significant elevation noticed in the levels of serum diagnostic marker enzymes in group B antituberculosis drugs administered rats as compared to that of group A normal rats. Co-administration of onion extract and antituberculous drugs, lowered enzyme levels were noticed in a dose dependent manner in Group C and D rat's serum samples. Aqueous onion extract prevented the antituberculosis drugs induced rise in levels of these diagnostic marker enzyme in serum of group C and group D rats as compared to those group B rats, indicating cytoprotective effect of onion extract.

Comparison of ALT among four groups:

Biochemical Parameters	Group-A Mean ± S.E.M. n = 12	Group-B Mean ± S.E.M. n = 12	Group-C Mean ± S.E.M. n = 12	Group-D Mean ± S.E.M. n = 12	p Value
ALT u/l	53.40±5.60	89.00±5.50	50.36±4.16	43.00±3.29	<0. 01

Table 1:

The range of ALT was 27 – 114 U/L with a mean ALT of 58.35. Significant difference was observed in ALT level of four groups p <0.01. Significant difference was observed in ALT level of group B and group C p < 0.01 showing that ALT level was observed to be higher in group B than in group C (89.00±5.50Vs 50.36±4.16). Similarly group B was compared with group D and showed a significant difference p< 0.01 in ALT level ((89.00±5.50Vs 43.00±3.29). When normal group A was compared with group C and group D rats no significant difference was observed in ALT levels p = 0.967, p = 0.403 respectively



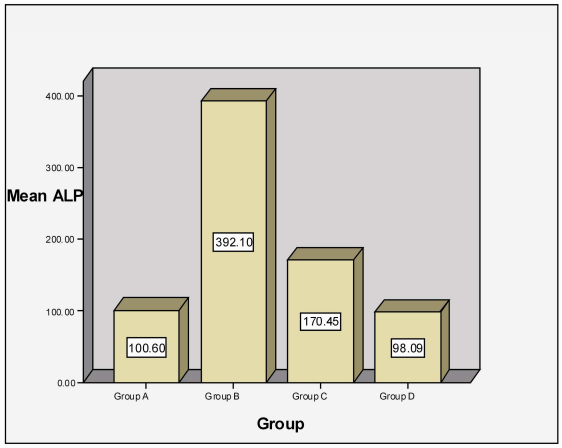
Comparison of ALP among four groups:

Biochemical Parameters	Group-A Mean ± S.E.M. n = 12	Group-B Mean ± S.E.M. n = 12	Group-C Mean ± S.E.M. n = 12	Group-D Mean ± S.E.M. n = 12	P Value
ALP u/l	100.60±12.57	392±.18.34	170.45±7.74	98.09±8.90	<0.01

Table 2:

The range of ALP was 42- 463 mean ALP of 187.0. Significant difference was observed in ALP level of four groups p <0.01.Significant difference was observed in ALP level of group B and group C p < 0.01 showing that ALP level was observed to be higher in group B than in group C (392±.18.34 Vs 170.45±7.74)

. Similarly group B was compared with group D and showed a significant difference p< 0.01 in ALP level (392±.18.34 Vs 98.09±8.90). When normal group A was compared with group C the change in ALP is significant p = 0.01 and group D rats showed no significant change in ALP p = 0.999.



DISCUSSION

Drugs used for the treatment of tuberculosis infection frequently induce acute liver injury. Typically, drug induced hepatotoxicity is assessed mainly by measuring serum levels of total billirubin and activity of aminotransferases. In general, the increased values of all these three parameters are associated with and reflect the status of liver injury, although in many cases of liver injury, they remain unchanged.

Antibiotic related liver injuries cover most of the clinical and pathological expressions of hepatic dysfunctions, including cytotoxic hepatitis. There is significant elevation observed in the levels of ALT, and ALP in the serum of antituberculous drugs administered rats (Group B) as compared to non-antituberculous drugs administered rats (Group A). Elevated levels of these enzymes in serum are presumptive markers of drug induced necrotic lesions in the hepatocytes. Enhanced susceptibility of hepatocytes cell membrane to antituberculous drugs induced peroxidative damage might have resulted in increased release of these diagnostic marker enzyme level into the systemic circulation.[14]

Previous studies have shown that micro constituents of *Allium cepa linn (onion)*, such as flavonols (quercetin) or organosulphur compounds (polysulphides), were able to modify the levels of phase 1 and phase 2 enzymes when administered in isolation. [15] There is evidence that *Allium cepa linn* or onion extract decreases levels of CYT P450 E1, The inhibition of P450 E1 plays an important role in onion extract induced hepatoprotection against toxicity of xenobiotics by acting as a chemopreventive agent by decreasing metabolic activation. In acetaminophen induced hepatotoxicity 10-100mg/kg of aqueous onion extract showed a significant correction of metabolic enzyme levels of liver.The activity of ALT and AST are sensitive indicators of acute hepatic necrosis, and the ALP level is known to be indicative of hepatobilliary disease.[16]

In the present study, co-administration of onion extract and antituberculous drugs maintained the levels of these diagnostic marker enzymes in the serum of Group C and Group D animals towards normal as compared to Group B rats, indicating the cytoprotective and antioxidant effect of onion aqueous extract. The comparison of mean values of ALT in all groups indicates antituberculosis drugs induced rise in ALT and then protection of onion extract in a dose dependent manner. In normal group mean ALP with standard deviation was 100.60± 12.57 which raised to 392± 18.34 in control group rats which were treated with antituberculosis drugs Isoniazid, Rifampicin and Pyrazinamide attributing the damaged structural integrity of the liver parenchyma ALT, ALP are normally located in the cytoplasm and are released into the circulation after cellular damage. The co-administration of onion extract along with antituberculosis drugs, prevented drugs induced hepatotoxicity in a dose dependent manner decreasing ALP values to 170.45± 7.74 at 100mg/kg dose of aqueous onion extract and almost close to normal ALP value that is 98.09±8.90 at high dose 200mg/kg of onion extract This result shows a significant ($p<0.001$) difference. The co-administration of onion extract along with antituberculosis drugs, prevented drugs induced hepatotoxicity in a dose dependent manner decreasing ALT values to 50.36± 4.16 at 100mg/kg dose of aqueous onion extract and almost close to normal ALT value that is 43.00± 3.29at high dose 200mg/kg of onion extract This result shows a significant ($p<0.001$) difference.

CONCLUSION

The results of the present study indicate that the co-treatment of onion extract and antituberculous drugs prevents antituberculosis drug's induced hepatotoxicity in rats, and kept the marker enzymes in normal level.

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