Biological effects of antioxidant enzymes in rat liver homogenate on drug treatment

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Abstract

Antibiotics are considered as a common cause of drug-induced liver injury (DILI) which is a leading health problem especially in a globally expanding commercialization of new drugs and the increasing exposure of patients to new compounds. This is expected to increase because of the number of drugs being consumed, prescription and non-prescription, as well as because of the current tendency towards pharmacologically active complementary and alternative medicines, dietary supplements, recreational substances and special diets. Liver is the major site of xenobiotic accumulation and biotransformation, analysis of initial molecular lesions elicited by pollutants in this organ result in early–warning and sensitive detection of chemical–induced carcinogenesis lesions. Endoplasmic reticulum of liver cells contain enzymes of the cytochrome P-450 monooxygenase system responsible for the conversion of chemicals to more polar metabolites that can be further conjugated to make urinary and biliary excretion attainable. The liver damage due to amoxicillin administration resulted in elevation in the activities of serum transaminase and $\gamma$-glutamyl transpeptidase. DILI is the primary adverse event that results in the withdrawal of drugs from the market and a frequent reason for the failure of drug candidates in the pre-clinical or clinical phases of drug development. Molecular analysis of the rat liver and plasma samples, combined with statistical analysis, revealed many similarities and differences between the in vivo biochemical effects of the drug.

Key words: Molecular markers, Drug-induced liver injury (DILI), hepatotoxicity, antioxidant enzymes, xenobiotics, Hepatocellular carcinoma,

Introduction

The chemical properties of antibiotics

Biochemical markers of chemical exposure include enzymes, proteins and other macromolecules associated with physiological functions (Bhaskar, 2015). Molecular markers commonly used in monitoring programmes include stress proteins (Hsp), cytochromes, P450 monooxygenases, Phase-II conjugating enzymes, heme porphyrin systems, metallothioneine and responses associated with reactive oxidants. Chemical structures that are recognized as increasing the ability to form reactive metabolites or exert direct toxic effects. Thus ebrotidine and famotidine, H2-receptor antagonists, have the same thiazole ring, but the former differs in that the side chain bears a 4-bromo-benzene ring and has a recognized hepatotoxic potential. Similarly, temafloxacin and trovafloxacin share a unique difluorinated side chain that is not found in the other quinolones, and which renders these drugs highly lipophilic. Clontiazepam and bentazepam are two thienodiazepine derivatives with a tricyclic structure which share a thiophene ring making the molecule more structurally similar to phenothiazines than to other benzodiazepines. Both drugs have led to several instances of moderate to severe hepatitis and even chronic liver injury. Many xenobiotics are capable of causing some degree of liver injury. The rat animals in the experimental groups were treated for drugs and chemicals (xenobiotics) may affect liver function which stimulate the activity of microsomal enzymes (eg., cyt.P450), a process known as enzyme induction. This is important in determining the degree of hepatotoxicity in the animal study (Conney, 1967).

PATHOLOGY OF MAJOR ANTIBIOTICS

Anti-hypermetric drugs consist of asymptomatic and often transient rise of serum transaminase and according to various reports in patients receiving drug (Kshirsagar, 2008). Drugs and other exogenous compounds may affect the liver in various ways.
The anti-y to -Several anti-erivatives: Phenylbutazone, Oxyphenbutazone Oxicams: Isoxicam, Sudoxicam. -te hepatitis; they most often cause

NRH is a rare condition characterized by apparent no -ntially hepatotoxic, when given in combination, their toxic effect is enhanced.

Isoniazid: The CYP2E1 c1/c1 genotype is associated with a higher CYP2E1 activity and may lead to a higher production of hepatotoxins.

Pyrazinamide: Pyrazinamide (PZA; pyrazoic acid amide) is converted to pyrazinoic acid and further oxidized to 5-hydroxyoxypyrarzinoic acid by xanthine oxidase. The serum half-life of pyrazinamide is not related to the length of treatment, indicating that pyrazinamide does not induce the enzymes responsible for its metabolism. Enzymes are involved in pyrazinamidetoxicity and whether toxicity is caused by pyrazinamide or its metabolites.

It is the most important cause of the drug induced toxic injury to several organ systems, including well known injury to gastrointestinal tract and kidneys. The epidemiological risk of clinically apparent liver injury is low (1–8 cases per 100 000 patient years use), but when it occurs, it can be serious and can cause diagnostic confusion (Sgro et al., 2002). Nearly all of the NSAIDs (NonSteroidal Anti Inflammatory Drugs) have been implicated in causing liver injury, and tend to be hepatocellular in nature: the mechanism is thought to be immunological idiosyncrasy (Zimmerman, 1990). Several Non-Steroidal Anti Inflammatory Drugs have been withdrawn from clinical use because of associated hepatotoxicity (Connor et al., 2003). The new more selective COX-2 inhibitors (eg. celecoxib, rofecoxib, nimesulide) are also associated with hepatotoxicity (Benichou, 1990).


Diclofenac: Diclofenac hepatotoxicity is an archetype of idiosyncratic DILI (Drug induced liver injury) (Mitchell et al., 1973). About 15% of those patients regularly taking diclofenac develop elevated levels of liver enzymes, and a threefold rise in transaminase levels has been reported in 5%. Accumulation of reactive metabolites generates oxidative stress and mitochondrial permeability transition, leading to cellular injury. In addition, covalent binding of reactive metabolites to ‘self’ proteins results in the formation of neoantigens.

Sulindac: Sulindac has been the drug most consistently associated with hepatotoxicity. Liver injury leads to impairment of bile flow and cases are predominated by itching and jaundice, 25% a hepatocellular pattern and the rest a mixed pattern of injury (Tarazi et al., 1993).

Anti-Retrovirals: Several anti-retrovirals have been reported to cause fatal acute hepatitis; they most often cause asymptomatic elevations of transaminases. Liver toxicity is more frequent among subjects with chronic hepatitis C and/or B.

Protease inhibitors: Include Ritonavir, Indinavir, Saquinavir and Nelfinavir. Hepatotoxicity became more evident after the introduction of ART (Anti-retrovirals) of high activity, which initially included invariably a protease inhibitor (PI).

Azathioprine-related NRH (Nodular regenerative hyperplasia): NRH is a rare condition characterized by apparent nodularity caused by variation in the size of liver cell plates; some plates are more than one cell thick, whereas others appear thinned and atrophic. NRH is caused by alterations in blood flow associated with obliterative changes within intra-hepatic portal radicals. Atrophic areas represent acini with decreased bloodflow, and nodular areas represent hypertrophic response. Thiouguaine, a metabolite of azathioprine, has been implicated in vascular injury associated with lying down of collagen in the space of Disse that lies between hepatocytes and sinusoidal endothelial cells.

(Kshirsagar, 2009) and the examination of blood and liver become significant to help in the diagnosis and treatment of the many diseases including cancer (Bhaskar, 2012).

Anti-Tubercular Drugs: The anti-tubercular drugs namely, Rifampicin, Isoniazid and Pyrazinamide are potentially hepatotoxic drugs which are metabolized by the liver. Adverse effects of antitubercular therapy are sometimes potentiated by multiple drug regimens. Thus, though INH, Rifampicin and Pyrazinamide each in itself are potentially hepatotoxic, when given in combination, their toxic effect is enhanced.

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Phenytoin: Phenytoin hepatotoxicity is a serious idiosyncratic reaction that occurs in less than one percent of patients. The phenytoin hepatotoxicity can elevate the level of aminotransferases, lactic dehydrogenase, alkaline phosphatase, bilirubin, and prothrombin time in serum.

Figure 1: Chemical Structure of Amoxicillin

Amoxicillin is a semisynthetic aminopenicillin antibiotic structurally related to the penicillin family. Chemically amoxicillin is (2S,5R,6R)-6-(R)-2-amino-2(p-hydroxyphenyl)acetamido-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[4.3.2]heptanes-2-carboxylic acid. There are similar structural analogs including ampicillin which provides similar function. The moderate-spectrum antibiotic is effective against a wide range of Gram-positive bacteria and a limited number of Gram-negative microbes.

The medicine is cheaper and safer as proved by more than forty years of research studies. Allergies are common and the drug is more likely to result in diarrhoea as side effect. It is not effective against bacterial species producing beta lactamase enzyme. A recent study has found a correlation between tooth enamel defects and increased use of amoxicillin infancy. The combination of Amoxicillin and Clavulanic acid is an oral antibacterial combination consisting of the semisynthetic antibiotic amoxicillin and the β-lactamase inhibitor, clavulanate potassium. Amoxicillin is the analogue of ampicillin, derived from the basic penicillin nucleus 6-amino penicillinic acid. Molecular weight of amoxicillin is 419.46.

Efficacy: Amoxicillin is more effective and acts against a wide range of pathogenic microbes.

Penetration into tissues: Amoxicillin penetrates better into tissues than penicillin. The only exceptions are brain tissues and spinal fluid.

Safety: Both are suitable for use in pregnancy and in paediatrics.

Cost: Both antibiotics are cheaper and are available in generic formulations.

Duration of treatment: Treatment with Amoxicillin requires fewer courses of antibiotics compared to Penicillin.

Pharmacokinetics

Amoxicillin serum concentrations achieved with the serum concentrations of Amoxicillin and clavulanic most commonly that equivalent to those produced by the oral administration alone. Amoxicillin diffuses readily into most body tissues and fluids with the exception of the brain and spinal cord. Hepatocellular carcinoma is a cancer arising from the liver. It is also known as primary liver cancer or hepatoma. The liver is made up of different cell types (e.g., bile ducts, blood vessels, and fat-storing cells). However, liver cells (hepatocytes) make up 80% of the liver tissue. Thus, the majority of primary liver cancers (over 90 to 95%) arises from liver cells and is called hepatocellular cancer or carcinoma. In the liver, alkyl pesticides are known to induce a large number of enzymes, such as alkaline phosphatase, that can serve as indicators of biological responses when observing the toxicity of these substances. Key enzyme systems include cytochrome P450 monooxygenases, phosphoesterases, glutathione-S-transferases, and O-alkyl and O-aryl conjugation.

Liver abnormal Conditions

- **Hepatitis**: Inflammation of the liver, usually caused by viruses like hepatitis A, B, and C. Hepatitis can have
non-infectious causes too, including heavy drinking, drugs, allergic reactions, or obesity.

- **Cirrhosis**: Long-term damage to the liver from any cause can lead to permanent scarring, called cirrhosis. The liver then becomes unable to function well.
- **Liver cancer**: The most common type of liver cancer, hepatocellular carcinoma, almost always occurs after cirrhosis.
- **Liver failure**: Liver failure has many causes including infection, genetic diseases, and excessive alcohol.
- **Ascites**: As cirrhosis results, the liver leaks fluid (ascites) into the belly, which becomes distended and heavy.
- **Gallstones**: If a gallstone becomes stuck in the bile duct draining the liver, hepatitis and bile duct infection (cholangitis).
- **Hemochromatosis**: Hemochromatosis allows iron to deposit in the liver, damaging it. The iron also deposits throughout the body, causing multiple other health problems.
- **Primary sclerosing cholangitis**: A rare disease with unknown causes, primary sclerosing cholangitis causes inflammation and scarring in the bile ducts in the liver.
- **Primary biliary cirrhosis**: In this rare disorder, an unclear process slowly destroys the bile ducts in the liver and permanent liver scarring (cirrhosis).

Liver injury is extremely rare with ampicillin, and rare with benzylpenicillin (penicillin G) and phenoxy methylpenicillin (penicillin V). Transient increases in ALT have been reported with oxacillin, carbenicillin and ticarcillin. Chang and Schiano (2007) identified Amoxicillin-clavulanic acid is one of the most frequently implicated causes of drug-induced liver injury worldwide. Padma et al., (1998) studied that Rifampicin also interacts with antiretroviral drugs and affects the plasma levels of these drugs as well as risk of hepatotoxicity. To have better understanding of the selective toxicity of these compounds, laboratory of Acosta et al.,(1985) has developed primary culture systems of liver, heart and kidney cells as experimental model to study the cellular and subcellular toxicity of selected antibiotics. The hepatotoxicity of therapeutic agents and pharmacutical chemicals has become an area of intense research interest. FDA (1983) and Taggart and Alderice (1982) revealed Benoxaprofen was removed from prescription drug when clinical evidences of its hepatotoxicity.

**Experimental design of antibiotic therapy**

Basic parameters of rat liver / 250 g -- Weight :-10.00 g , Volume :-19.60 ml & Bile flow:-22.5 ml

1. Animals and grouping of animals
   - **Group-I** - C-Control treated with normal feed.
   - **Group-II** – T1-Rats fed with 0.200 mg per kg Amoxicillin
   - **Group-III** – T2-Rats fed with 0.400 mg per kg Amoxicillin
   - **Group-IV** – T3-Rats fed with 0.600 mg per kg Amoxicillin
   - **Group-V** – T4 -Rats fed with 0.800 mg per kg Amoxicillin
   - **Group-VI** – T5-Rats fed with 1.000 mg per kg Amoxicillin

After treatment of specific period,animals were kept starved overnight on 14th day,29th day,44th day,59th day, 74th day and 89th day of experiments. On next day the animals were sacrificed by decapitation and the liver in each case were dissected out and washed with saline. Biochemical parameters require 1.5 to 50 µ l serum or plasma and are pre-programmed with as many as 20 different clinical chemistry tests. The challenge of clinical pathology laboratory was to develop or adapt procedures designed for human use into reliable tests for the elevation of organ function and toxicity.

2. Preparation of liver homogenate
The liver tissue was ground in cold mortar and pestle or homogenizer. Homogenate was kept for 15 minutes under cold conditions and collected the clear homogenate from the top for studies.

Homogenised about 400 mg of tissue in minimum (5 ml) ice-cold buffer (0.05M Sucrose)

Centrifuged at 10000 rpm for 5 minutes

Collected the clear supernatant

Added 90% ammoniumsulfate crystals so that protein was precipitated

Centrifuged and collected the precipitate

precipitate was dissolved in Tris buffer

The above solution was taken in dialysis bag and dialysed overnight in Tris-buffer (0.0025 M; pH 7.4)

Supernatant take out as the enzyme source (Beutler, 1986)

3. Antioxidant enzymes in liver

- Glutathione-s-Transferase (E.C.2.5.1.8) (Beutler, 1986)

**Principle**
The interaction of foreign compounds with GSH is catalysed by GST and the formation of GSH conjugates. Here GST catalyse the interaction of CDNB and GSH resulting in glutathione conjugates. The change in absorbance can be recorded at 340 nm. The principle of GST catalysed reaction equated as CDNB+GSH→CDNB-s-Glutathione.

**Reagents**
- a) Potassium phosphate buffer (K2HPO4/KH2PO4, 0.5M, 25mM, pH 6.5)
- b) CDNB in 95% ethanol, 25 mM
- c) Reduced glutathione, 20 mM

**Procedure:** Reagent ‘a’ (200µl) and reagent ‘b’ (20µl) were added to two tubes named blank and test. 730 µl of distilled water was added to blank and add 680 µl of distilled water was added to the system and both the tubes were incubated at 37°C for 10 minutes. Afterwards added 50 µl of reagent ‘c’ to both the tubes and mixed well. 50 µl of hemolysate was added to the system and the change in absorbance was recorded at 340 nm.

**Calculation**

\[ A = \frac{\Delta \text{OD}}{\sum N} \]

Where \( \sum = 9.6 \), N=1

b) Glutathione reductase (EC 1.8.1.10) (Beutler, 1986)

**Principle**

\[ \text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow 2 \text{GSH} + \text{NADP}^+ \]

**Reagents**
- a) Tris-HCl, 1M, EDTA (5mM), pH 8
- b) Oxidised glutathione (0.033M) (neutral)
- c) NADPH (2mM)

**Assay**
The reaction mixture contained 50 µl Tris-HCl EDTA buffer and 10 µl 1:20 hemolysate. Also added 890 and 790 µl of distilled water to blank and tests respectively. Both the tubes were incubated at 37°C for 10 minutes. After incubation added 100 µl oxidized glutathione to the test and both the tubes were reincubated at 37°C for 10 minutes. 50 µl NADPH was added to both tubes and change the OD was recorded at 340 nm.

\[ \Delta \text{OD} = \frac{\sum \times N \times \text{VH}}{\sum} = 6.22 \]

\[ \text{VH} = 0.01 \]

(c) Glutathione peroxidase (EC 1.11.1.9) (Rotruck, 1984)

**Reagents**

- Tris HCl - 0.4 M, pH-7.0
- Sodium azide - 10 mM
- TCA - 10%
- EDTA - 0.4 mM
- H\(_2\)O\(_2\) - 0.2 mM
- GSH solution - 2 mM

**Procedure**

To 0.2 ml of Tris buffer, 0.2 ml EDTA, 0.1 ml Sodium azide and 0.5 ml sample homogenate, were added and mixed well. To this mixture 0.2 ml of GSH followed by 0.1 ml of H\(_2\)O\(_2\) solution were added. The contents were mixed well and incubated at 37°C for 10 minutes with a control containing all reagents except tissue homogenate. After the reaction was arrested by the addition of 0.5 ml of 10% TCA. Tubes were centrifuged and supernatant was assayed for GSH.

**GSH estimation**

- Phosphate buffer - 0.2 M, pH - 8
- TCA - 5%

Ellman’s reagent - 19.8 mg DTNB/100 ml of 0.1% Sodium citrate

Standard glutathione solution = 10 mg GSH/100 ML H\(_2\)O

1 ml of the supernatant from above test tubes was taken. 0.5 ml of Ellmann’s reagent and 3 ml of phosphate buffer were added. The yellow color developed was read at 412 nm.

4. Lipid peroxidation

(a) TBARS (Thio Barbituricacid Reactive Substances) (Nichans & Samuelson, 1968)

**Reagents**

- 0.25M Tris HCl buffer (pH – 7.5)
- 15% w/v Trichloroacetic acid
- 0.375 w/v Thio barbituric acid in 0.25 N HCl
- TCA-TBA HCl reagent. Mix the reagents 2&3 in equal volume.

**Procedure**

1 ml sample homogenate was combined with 2 ml of TCA-TBA HCl reagent & mixed thoroughly. The solution was then heated on a water bath for 15 minutes. The resultant flocculant precipitate was removed by centrifuged at 10000 g for 15 minutes. The absorbance of supernatant was read at 535 nm against a blank solution that contain no sample extract. The concentration of TBARS was determined using a molar extinction coefficient of 1.56 x 10\(^7\)/m/cm.
Results

Figure-4: Rat liver after six months of antibiotic treatment

The Values are average for six rats in each group and are expressed in grams ± SEM.

Group-1 Control, Group-2 to 6 are tests. a - Statistical difference with control group at P < 0.05

b - Statistical difference with test group at P < 0.05

<table>
<thead>
<tr>
<th>Group</th>
<th>Glutathione–S-transferase</th>
<th>Reduced Glutathione (µ g/liver)</th>
<th>Lipid Peroxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal-C</td>
<td>1909.5 ± 415.5 a</td>
<td>9321.3 ± 683.3 a</td>
<td>86.0 ± 14.50 a</td>
</tr>
<tr>
<td>Group-2 T1</td>
<td>1539.3 ± 425.4 b</td>
<td>8246.5 ± 862.8 b</td>
<td>82.2 ± 36.30 b</td>
</tr>
<tr>
<td>Group-3 T2</td>
<td>1449.3 ± 535.4 b</td>
<td>8546.5 ± 682.6 b</td>
<td>87.2 ± 26.30 b</td>
</tr>
<tr>
<td>Group-4 T3</td>
<td>1265.3 ± 565.4 b</td>
<td>7846.5 ± 782.6 b</td>
<td>82.2 ± 36.3 b</td>
</tr>
<tr>
<td>Group-5 T4</td>
<td>1345.3 ± 145.5 b</td>
<td>7815.7 ± 872.6 b</td>
<td>77.2 ± 36.3 b</td>
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<tr>
<td>Group-6 T5</td>
<td>1349.3 ± 245.4 b</td>
<td>7456.6 ± 862.6 b</td>
<td>77.2 ± 36.3 b</td>
</tr>
</tbody>
</table>

Table-1 and Graph-1 Antioxidant enzymes in liver homogenate after 15 days of treatment

(In mol product/min/mg)

Significance: There was an increase in Glutathione in all the groups or extend when compared to the normal.

<table>
<thead>
<tr>
<th>Group</th>
<th>Glutathione–S-transferase</th>
<th>Reduced Glutathione (µ g/liver)</th>
<th>Lipid Peroxidase</th>
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</thead>
<tbody>
<tr>
<td>Normal-C</td>
<td>1909.5 ± 45.5 a</td>
<td>9321.3 ± 683.3 a</td>
<td>86.0 ± 14.50 a</td>
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<tr>
<td>Group-2 T1</td>
<td>1449.3 ± 45.4 b</td>
<td>7546.5 ± 842.6 b</td>
<td>97.2 ± 36.3 b</td>
</tr>
<tr>
<td>Group-3 T2</td>
<td>1649.3 ± 45.4 b</td>
<td>7567.5 ± 832.6 b</td>
<td>99.2 ± 46.3 b</td>
</tr>
<tr>
<td>Group-4 T3</td>
<td>1549.3 ± 45.4 b</td>
<td>7568.5 ± 892.6 b</td>
<td>97.2 ± 46.3 b</td>
</tr>
<tr>
<td>Group-5 T4</td>
<td>1657.3 ± 45.4 b</td>
<td>7567.5 ± 892.6 b</td>
<td>97.2 ± 46.3 b</td>
</tr>
<tr>
<td>Group-6 T5</td>
<td>1849.3 ± 45.4 b</td>
<td>7578.5 ± 982.6 b</td>
<td>98.2 ± 46.3 b</td>
</tr>
</tbody>
</table>

Table-2 and Graph-2 Antioxidant enzymes in liver homogenate after 30 days of treatment

Significance: There was an increase in Glutathione in all the groups or extend when compared to the normal.
which was found to be significant.

<table>
<thead>
<tr>
<th>Group-1 Normal-C</th>
<th>Glutathione–S-transferase</th>
<th>Reduced Glutathione (µ g/liver)</th>
<th>Lipid Peroxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-2 T1</td>
<td>1909.5 ±41.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9321.3±683.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.0 ±14.50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group-3 T2</td>
<td>1849.3±45.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8546.5±882.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>97.2 ±36.3&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Group-4 T3</td>
<td>1859.3±45.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8536.5±872.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>97.4 ±36.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group-5 T4</td>
<td>1949.3±45.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8666.5±862.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98.6 ±30.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group-6 T5</td>
<td>1898.3±45.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8576.5±882.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>94.6 ±26.3&lt;sup&gt;b&lt;/sup&gt;</td>
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Table – 3 and Graph-3 Antioxidant enzymes in liver homogenate after 45 days of treatment
Significance: - There was an increase in Glutathione in all the groups or extend when compared to the normal which was found to be significant.

<table>
<thead>
<tr>
<th>Group-1 Normal-C</th>
<th>Glutathione–S-transferase</th>
<th>Reduced Glutathione (µ g/liver)</th>
<th>Lipid Peroxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-2 T1</td>
<td>1869.3±45.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8346.5±862.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.2 ±36.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group-3 T2</td>
<td>1840.3±46.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8576.5±882.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>86.2 ±36.3&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Group-4 T3</td>
<td>1810.3±44.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8646.5±862.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>86.2 ±36.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group-5 T4</td>
<td>1829.3±45.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8666.5±862.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.3 ±36.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group-6 T5</td>
<td>1832.3±45.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8646.5±802.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>86.2 ±86.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table – 4 and Graph-4 Antioxidant enzymes in liver homogenate after 60 days of treatment
Significance: - There was an increase in Glutathione in all the groups or extend when compared to the normal which was found to be significant.

<table>
<thead>
<tr>
<th>Group-1 Normal-C</th>
<th>Glutathione–S-transferase</th>
<th>Reduced Glutathione (µ g/liver)</th>
<th>Lipid Peroxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-2 T1</td>
<td>1449.3±45.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8547.5±882.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.2 ±36.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group-3 T2</td>
<td>1649.3±45.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8648.5±882.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.2 ±30.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group-4 T3</td>
<td>1749.3±44.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8666.5±862.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88.2 ±36.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group-5 T4</td>
<td>1829.3±45.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8548.5±862.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>86.2 ±36.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group-6 T5</td>
<td>1869.3±46.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8646.5±892.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.2 ±36.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table – 5 and Graph-5 Antioxidant enzymes in liver homogenate after 75 days of treatment
Significance: - There was an increase in Glutathione in all the groups or extend when compared to the normal which was found to be significant.

<table>
<thead>
<tr>
<th>Group-1 Normal-C</th>
<th>Glutathione–S-transferase</th>
<th>Reduced Glutathione (µ g/liver)</th>
<th>Lipid Peroxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-2 T1</td>
<td>1909.5±45.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9321.3±683.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.0±14.50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Significance: There was an increase in Glutathione in all the groups or extend when compared to the normal which was found to be significant.

Conclusion

Administration of drugs in patients underlying liver diseases involves a balanced assessment of risk benefits. Genetic polymorphism among enzymes involved in drug metabolism account for drug hepatotoxicity. Antibiotics are commonly implicated cause of DILI while hepatotoxicity is one of the most important adverse drug reactions associated with drugs that may limit their use (Bhaskar, 2016). Amoxicillin used in this hepatotoxicity study is the most frequently implicated antibiotics. There is the study of antioxidant enzymes like Glutathione-S-transferase, reduced glutathione and lipid peroxidase in mol product / min /mg protein. This studies were done for a period of 15 days, 30 days, 45 days, 60 days, 75 days and 90 days approximately. GST are a group of malfunctional proteins localized mainly in the hepatic cytosol and participate in detoxification reactions. Toxicity signs were observed in the experimental animals within the observation period. Drug induced liver injury covers a variety of types and includes most of the clinical and pathological expressions of liver damage (Zimmerson, 1974). In present study glutathione level were found to be decrease significantly in the liver. Glutathione – S-Transferase utilizes as a co-factor and therefore change observed in its ability after treatment. Antioxidant enzymes in the liver homogenate in mol product/ min/mg protein varied in the experimental tests of GST, GSSG and lipid peroxidase after the final treatment. Target organ toxicology has become an important discipline because of the increased interest in the specificity of toxicity of xenobiotics to key issues of the body.

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References


Table 6 and Graph 6 Antioxidant enzymes in liver homogenate after 90 days of treatment


