ORIGINAL ARTICLE

# EFFECT OF AZADIRACHTICA INDICA LEAVES AQUEOUS EXTRACT ON ERYTHROMYCIN INDUCED HEPATIC DAMAGE

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**ABSTRACT**

**Background:** Erythromycin, a commonly used antibiotic for various respiratory tract infections, is well documented for its hepatotoxic effect, which is probably due to the oxidative stress produced by this drug. Azadirachtica Indica, commonly known as Neem is a rich source of various bioactive compounds and has shown strong antioxidant effect in various researches. This study was designed to find out the effect of aqueous extract of Neem leaves on liver enzymes; Alanine transaminase (ALT) and Aspartate transaminase (AST) against liver damage caused by erythromycin.

**Methods:** This study was conducted in Baqai Medical University, Karachi in 2017 spanning a period of 6 months. Eighty male albino wistar rats were taken randomly and were divided into 4 groups of 20 animals each; A(control), B (received erythromycin 100mg/kg body weight), C (received erythromycin 100mg/kg body weight plus aqueous Neem Extract at the dose of 500mg/kg body weight) and "D" (received only aqueous Neem Extract at 500mg/kg body weight). After 14 days of continuous treatment, rats were sacrificed and the blood samples were collected via cardiac puncture and then sent to the laboratory for the investigation of liver enzymes ALT and AST using standard reagent kits.

**Results:** Serum ALT and AST enzymes were found to be decreased in group B and C. The results were Statistically significant.

**Conclusion:** Azadirachtica Indica aqueous extract showed protective effect on erythromycin induced hepatic damage.

**Keywords:** Azadirachtica Indica, Aqueous extract, Erythromycin, Aspartate transaminase, Alanine transaminase, Hepatoprotective.

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## INTRODUCTION

Liver, the major organ in our body which performs a variety of functions in metabolism, biosynthesis, toxins clearance and elimination of drugs including antibiotics such as erythromycin.[1]

Erythromycin belongs to the macrolide family of antibiotics, generated from Streptomyces erythraeus (saccharopolyspora erythraea). It was first discovered in 1919 by Waksman. Later different strains were obtained from the isolate.[2] Erythromycin is a drug of choice for ENT physicians in their Out Door Patient Departments[3] for treating bronchitis, pneumonia, pertussis and diphtheria. Erythromycin a known pro-kinetic (cholecystokinetic) drug is one of the most commonly used macrolides prescribed in many countries for respiratory tract infections.[4,5] It can be used in patients suffering from chronic obstructive pulmonary disease (COPD) where it can decrease exacerbations and reduce airway inflammation. It is also useful in the management of chronic patients especially for Diffuse Pan Bronchiolitis (DPB), a

non-infectious chronic lung inflammation where long term treatment is required for treating such patients. Treatment with erythromycin has been documented to reduce the levels of interleukin-8 (IL-8) protein and the number of neutrophils in fluid taken from lungs from patients suffering from cystic fibrosis and Diffuse Pan Bronchiolitis (DPB).[10]

Erythromycin causes liver damage which is evident with the rise in liver enzymes i.e. alanine aminotransferase (ALT), aspartate aminotransferase (AST).[5] The calculated risk of significant cholestatic jaundice associated with the use of erythromycin is about 3.6 per 100,000 users.[3,4] Erythromycin at the dose of 100 mg/kg body weight deposits in several chief organs for example liver, heart, kidney and various glands. In addition, long term therapy of the drug erythromycin has hazardous consequences on liver causing hepatotoxicity, this occurs as a result of generation of reactive oxygen species .[11]

Azadirachta indica commonly known as Neem is a tree which has been used for centuries in agriculture and medicine. The word Azadiracihta is derived from Persian, the meaning of which is "Noble tree". It was first time discovered by a scientist named "De Jussieu" who gave the taxonomic nomenclature.[12]

Neem leaves are comprised of multiple compounds e.g., tritepenoids (such as 6 alpha-hydroxi-azadira-dione and di-hydronimocinol), sesquiterpene lactone such as Azadirachtin limonoid and its derivatives, nimbinin and some of its derivatives include quercetin, B-sitoesterol. They also contain carbohydrates (22.9%), proteins (10%) minerals (9.8%), magnesium, resin, calcium and phosphorus.[13]

Azadirachtaindica (Neem) leaves extract has great remedial abilities. It reduces hepatocellular necrosis and consequently reverts liver toxicity by bringing the liver back to normal functions. It stimulates storage and production of proteins in the liver. Azadirachtaindica extract enhances immune stimulant activity, cellularity, augments the mononuclear phagocyte systems and confers hepato-protection as well. The hepatoprotective dose of Neem extract is 500mg/kg body weight, it reduces hepatocellular necrosis and consequently revert the liver toxicity followed by bringing back the liver to normal functions.[14]

The objective of the study was to assess the effect of aqueous extract of Neem leaves on liver enzymes; Alanine transaminase (ALT) and Aspartate transaminase (AST) against liver damage caused by erythromycin.

## METHODS

This experimental study was carried out at the Department of Anatomy, Baqai Medical University (BMU), Karachi, permitted and ratified by the Board of Advanced Studies and Research (BASR) and the ethical committee of University. The design of study was experimental. The duration of the study was 14 days.

Eighty (80) grown up Albino Wistar male rats of 13-14 weeks of age, weighted between 180 to 200g were procured from animal house of BMU. The animals were placed in plastic cages (5 animals in each cage) under strict conditions of temperature (22± 2°C) and humidity (50-60%) in an alternating 12-hourslight/dark cycle. Animals were fed with standard diet and water regularly. Guiding principles of National Institute of Health (NIH) were followed for handling and experimentation on animals (National Research Council, 2007). Acclimatization of animals for about 10 days was assured, prior to the start of study.

Erythromycin tablets (500mg) manufactued by Indus Pharma were purchased from medical store Malir Cantt, Karachi. Neem leaves collected from the grown Neem trees at Baqai Research Department (Baqai Medical University) and Aqueous Neem leaves extract was prepared under the supervision of senior scientific officer at Pakistan Council Scientific & Industiral Research (PCSIR), Karachi.

Neem extract was prepared by grounding dry leaves soaked in water for about seven-day time period. Evaporation took place after water bath. Lastly pure Neem extract of about 25 gms was attained.

Eighty male albino wistar rats were taken randomly and were divided into 4 groups of 20 animals each; Group A: This group was kept as control and received no intervention and was fed with normal diet. Group B: This group received erythromycin 100mg/kg body weight as a single dose daily by oral route for a period of 14 days feed via oro-gastric tube. Group C: Group C received erythromycin 100mg/kg body weight as a single dose and aqueous Neem Extract at the dose of 500mg/kg body weight simultaneously through gastric lavage for 14 days. Group D: This group received only aqueous Neem Extract at 500mg/kg body weight as a single dose through oro-gastric tube for 14 days. Neem extract and Erythromycin were given with the help of oro-gastric tube about 1 hour distinctly.

Samples of the blood were taken through cardiac puncture in order to estimate the hepatic enzymes levels such as ALT and AST. Blood samples were taken with 5cc syringes into already marked tubes that contain antisera for analysis of hepatic enzymes such as ALT and AST through Biochemistry Analyzer Selectra E.

Statistical analysis was done by SPSS (statistical package for social sciences) version 23. Mean and Standard deviation for enzymes were calculated .Quantitative measures were performed by applying one-way analysis of variance (ANOVA) with the post-hoc Tukeys test .Qualitative test were evaluated by 't test'.If P value is <0.05, it is considered to be significant with 95% confidence interval.

## RESULTS

In Group A (Control group) mean value of serum ALT in animals was found to be 32.04±11.93 IU/L. In Group B (Erythromycin Treated Group) mean value of ALT in animals was 114.49±11.81IU/L. There was noteworthy increase (p<0.01) in ALT levels of group B animals when compared to group A animals.

The mean value in ALT of group C (Erythromycin plus neem treated group) animals was found to be 38.70±2.70IU/L. There was insignificant increase (p>0.01) in ALT level of group C animals in comparison with group A, but significant decrease (p<0.01) of ALT of group C animals in comparison to group B animals. Mean value of ALT of group D (Neem treated group) was found to be 32.11±10.86 IU/L. There was insignificant increase (p=0.99) in ALT level of group D animals when compared with group A animals, but significant decrease (p<0.01) of ALT in group D when compared to group B and group C animals

After applying Post Hoc Tukey test as depicted in Table 2, significant P value was observed between group A and B, group B and C and between group B and D (Table 1 and Figure 1).

**Table 1: Mean Comparison of Alt Iu/L In Various Animal Groups (N=80)**

| Group | Treatment | ALT (IU/L) Mean ±SD | P- Value |
|---|---|---|---|
| Group A | Control | 32.04±11.93 | - |
| Group B | Erythromycin Treated | 114.49±11.81 | P<0.01 in comparison to group A |
| Group C | Erythromycin plus neem treated | 38.70±2.70 | P<0.01 in comparison to group B |
| Group D | Neem treated | 32.11±10.86 | P<0.01 in comparison to group B and C |

*Total No of rats in each group =20, Data is presented as mean ± standard deviation*

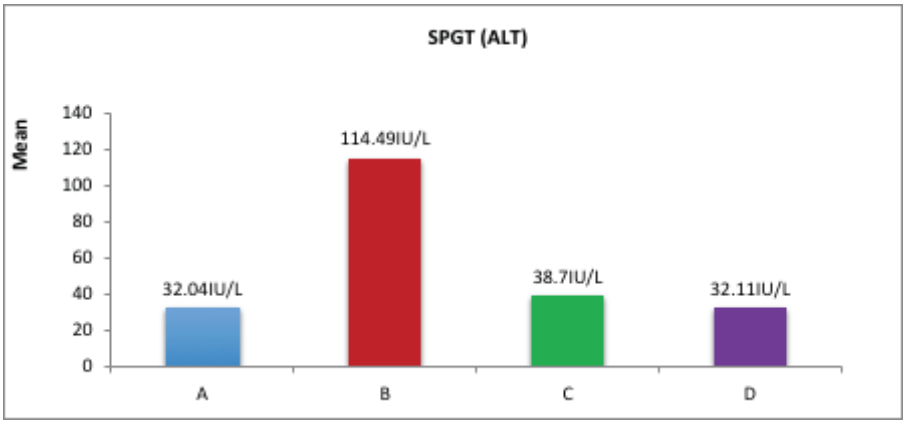


**Figure 1: Mean values of SGPT in A,B,C and D groups.**

**Table 2: Statistical Analysis of Serum Alt (Sgpt) (Iu/L) Levels of Rats between Different Study Groups (Post Hoc Tukey Test)**

| Comparison | Statistical Comparison | Difference of Means | p-value |
|---|---|---|---|
| **Group A and group B** | Negative Control and Treated | -82.45 | <0.01* |
| **Group A and group C** | Negative Control and Protected | -6.66 | .078 |
| **Group A and group D** | Negative Control and Positive Control | -0.07 | 1.000 |
| **Group B and group C** | Treated and Protected | 75.79 | <0.01* |
| **Group B and group D** | Treated and Positive Control | 82.38 | <0.01* |
| **Group C and group D** | Protected and Positive Control | 6.59 | .074 |

**P value < 0.05 considered significant**

*The effect on serum ast iu/l level were found statistically significant.*

Mean value of AST in group A (Control Group)animals was found to be 27.47±10.35IU/L. In Group B There was significant increase (p<0.01) in AST levels when compared with control group A animals. In Group C there was significant increase (p<0.01) in AST level when compared with control group A animals, but significant decrease (p<0.01) of AST of group C animals in comparison to group B animals. In Group D there was insignificant decrease (p=0.92) in AST level when compared with control group A animals, but significant decrease (p<0.01) of AST of group D animals when compared with group B and group C animals (Table 3 and Figure 2).

After applying Post Hoc Tukey test, significant P value was observed between group A and B, group B and C, group B and D and group C and D (Table 4).

**Table 3: Mean Comparison of AST Iu/L in Various Animal Groups.**

| Group | Treatment | AST(IU/L) Mean ±SD | P- value |
|---|---|---|---|
| Group A | Control | 27.47±10.35 | - |
| Group B | Erythromycin Treated | 112.8±20.63 | P<0.01 in comparison to group A |
| Group C | Erythromycin plus Neem treated | 34.81±4.27 | P<0.01 in comparison to group B |
| Group D | Neem treated | 26.26±10.21 | P<0.01 in comparison to group A and C |

*Total no of rats in each group=20, Data is presented as mean ± standard deviation*

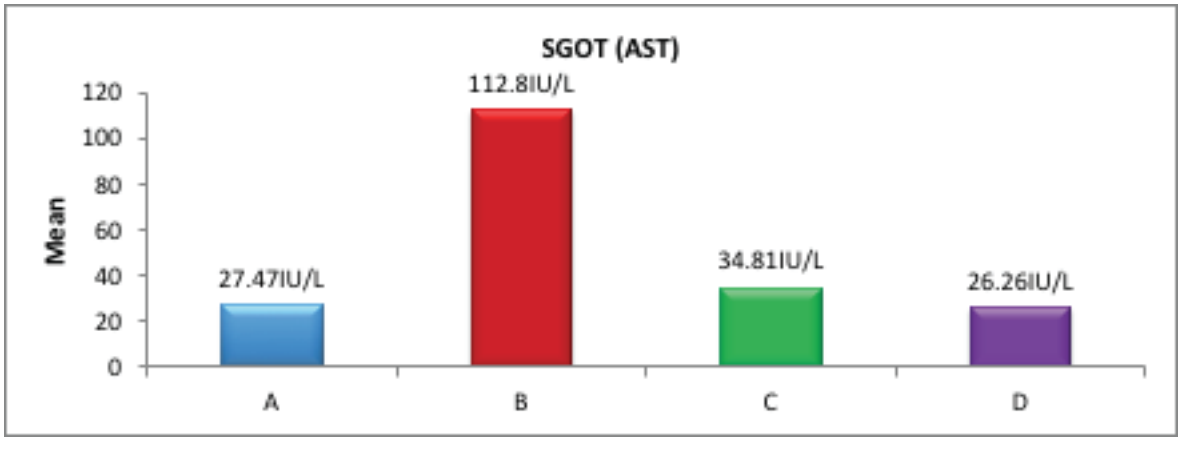


**Figure 2: This graph depicts Mean AST levels in A,B,C and D group.**

**Table 4: Statistical Comparison Analysis of Serum AST (Sgot) (Iu/L) Levels Of Rats among Different Groups.**

| Comparison | Statistical Comparison | Difference of Means | p-value |
|---|---|---|---|
| Group A and group B | Negative Control and Treated | -85.33 | <0.01* |
| Group A and group C | Negative Control and Protected | -7.34 | 0.042* |
| Group A and group D | Negative Control and Positive Control | 1.21 | 0.929 |
| Group B and group C | Treated and Protected | 77.99 | <0.01* |
| Group B and group D | Treated and Positive Control | 86.54 | <0.01* |
| Group C and group D | Protected and Positive Control | 8.55 | <0.01* |

**P value < 0.05 considered significant**

## DISCUSSION

Liver diseases are universal health issue affecting a large number of patients. Liver injuries can be due to intake of toxic chemicals, drugs, alcohol consumption and viral infections. Liver damage results in oxidative stress and redox imbalance in the body.[15]

In present study, erythromycin was given in the dose of 100mg/kg body weight to rats through oral route. This was in accordance to the dose administered by Skete et al. to experimental rats; he investigated the protective effect of luffa cylindrical linn against erythromycin induced toxicity. This was suggested due to the release of reactive oxygen species for instance superoxide anions and hydrogen peroxide. These free radicals initiate the process of lipid peroxidation and membrane degradation of liver cell plasma membrane.16In another study, it was found that erythromycin estolate, when given in a dose of 1500mg/kg produced hepatotoxicity.[17]

In contrast, Romeiro FG et al. in his experiment used erythromycin in a dose of 250 mg orally four times a day for the treatment of hepatic encephalopathy in cirrhotic patients. He concluded that erythromycin can accomplish two likely targets in liver cirrhotic patient that is, it maintain motility and transit time and thus decreases the overgrowth of intestinal bacterial. It also reduces the production of ammonia in colon as well as small intestine.[18,19]

In the present study, the serum levels of two important enzyme i.e., ALT, AST were measured which are frequently used as hepatobiliary biomarkers. Increased ALP and ALT levels indicate hepatocellular damage, whereas, increased AST activities indicate extensive necrosis and bile flow obstruction.[20]

In observations recorded in group B which was erythromycin treated group, serum alanine transaminase (ALT) range was noticeably raised compared to the animals of group A which was control group. The raised enzyme levels could be because of the necrosis and degeneration of hepatocytes resulting in discharge of transaminases into blood and also indicate raised permeability of cell membrane [22].

This is in accordance with the findings observed by Nilesh Mehta et al. who found the raised levels of ALT enzyme after hepatocellular injury in experimental animals after treatment with erythromycin. The author also documented raised ALT levels in paracetamol and carbamazepine induced hepatotoxicity. In contrast, Fernando Gomes Romeiro et al reported decrease in ALP enzyme levels in cirrhotic patients, when erythromycin was administered to them in the dose of 250mg/kg four times a day orally compared to the cirrhotic patients which were not given erythromycin.[23]

In group C animals which were administered erythromycin and Neem in combination showed significant decrease in hepatic ALT levels compared to the group B animals which were given erythromycin alone. This suggests that raised levels of liver enzyme ALT significantly reduced when compared with group B (erythromycin treated animals), but the levels did not approach that of normal levels. Neem leaves extract prevents components of the cell membranes and polyunsaturated fats from free radical oxidation and thus prevent the enzymes to release into the blood [24]. This is in agreement with the findings of Maruthappan V et al, who also found that Neem extract seems to decrease chemically induced liver injury in rats maintaining serum enzymes levels; he documented decreased levels of ALT with co administration of Neem extract in alcohol induced hepatotoxicity [25].

Present study also indicated noticeable rise in serum

AST levels in erythromycin treated group B animals as shown in (Table 3, Figure 2). This is in resemblance with the findings of George Aragon et al who found increased levels of ALP and AST. The enzyme AST is another marker of hepatic injury. Once hepatocytes are injured, both AST and ALT are discharged into blood in larger amounts[26,27]. Nassr-Allah H and N.Sambo also reported increase in the levels of enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in erythromycin treated rats and suggested that in the erythromycin toxicity, it causes hepatocytes to release ALT and ALP in blood.

In the present study, the levels of enzyme AST were reduced in animals treated with erythromycin and aqueous Neem leaves extract treated group C, but not reached up to normal levels. This was in agreement with the findings of Johnson et al., which observed decreased levels of AST and ALT in Acetaminophen induced hepatotoxicity in Sprague Dawley male rats. As AST and ALT are sensitive markers of necrotic lesions within the liver and their release into circulation is indicative of severe injury to hepatocyte membranes during paracetamol toxicity, when given with Neem extract the significant decrease was due to the antioxidant buffering capacity of Neem leaves which inhibits the paracetamol induced liver damage by decreasing reactive oxygen species, lipid, protein and DNA damage. Ajibade Adeshina John et al. also found that the Azadirachta indica likely exerted its hepatoprotective activity by acting as an antioxidant agent by inhibiting lipid peroxidation in paracetamol toxicity. There was a significant decrease in the hepatic enzymes AST and ALT after co-administration of Neem extract with paracetamol.

## CONCLUSION

Present study indicated protective effects of aqueous extract of Azadirachtica indica (Neem) on the erythromycin induced hepatic damage. The biochemical changes as depicted by liver enzymes AST and ALT were finely improved by Azadirachta indica (Neem). Aqueous Neem leaf extract is powerful antioxidant because of this it acts as a hepato-protective agent.

The limitations of the study were the scientific basis of prediction and extrapolation are examined in animals with special references to factors that lead to uncertainty in suggesting potential hazards or benefits in man. Further studies on large number of animals are required to assess the use of Neem leaves against erythromycin induced hepatotoxicity

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