Impact of Aqueous Neem Leave Extract on Erythromycin Induced Hepatotoxicity

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ABSTRACT

Objective: To examine the protective effect of aqueous neem leaves extract against damage induced by erythromycin on liver enzyme alkaline phosphatase.

Study Design: Laboratory-based experimental study.

Place and Duration of Study: Anatomy Department, Baqai Medical University, Karachi Pakistan, from Mar to Jun 2018.

Methodology: Eighty male albino wistar rats were taken and were divided randomly into 4 groups. Group-A served as a control group with no drug given. Group-B animals received the drug erythromycin 100 mg/kg body weight orally through gastric lavage. Group-C animals received erythromycin 100 mg/kg body weight and neem leaves extract in a dose of 500 mg/kg body weight orally through gastric lavage. Group-D received only neem extract at the dose of 500 mg/kg body weight orally. After 14 days, blood samples were collected via cardiac puncture and sent to laboratory for the investigation of alkaline phosphatase enzyme.

Results: The alkaline phosphatase enzyme levels of group-B (Erythromycin treated group) were found to be 176.98 ± 10.53 IU/L. When treated with aqueous neem leaves extract the alkaline phosphatase levels decreased to 150.10 ± 2.28 IU/L seen in group-C. The comparisons of group-B and group-C showed statistically significant ($p<0.01$), showing protective effect of aqueous neem leaves on hepatocytes.

Conclusion: Aqueous neem extract showed protective effect on erythromycin induced hepatic damage.

Keywords: Azadirachtica indica, Erythromycin, Hepatotoxicity.

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INTRODUCTION

The liver is significant organ in our body and plays out variety of functions, for example, digestion, biosynthesis, detoxifying the poisonous substances.[1,2]

The erythromycin drug belongs to the macrolide group of anti-infection agents, obtained from Streptomyces erythraeus (Saccharopolyspora erythraea). It was first discovered in 1919 by Waksman.[3] One of the adverse effects of erythromycin is liver toxicity. Possibility of major cholestatic jaundice linked with erythromycin use, was found to be around 3.6 for every 100,000 users.[3] Liver damage leads to elevated level of various enzymes of liver i.e. alkaline phosphatase (ALP), alanine transaminase (ALT) and alkaline transaminase (AST).[3] Erythromycin is a medication of choice for ENT doctors.[4] Erythromycin is a known prokinetic (cholecystokinetic) drug,[5] and standout amongst the most utilized macrolide agent for respiratory tract diseases.[6] When used in the patients experiencing chronic obstructive pulmonary disease (COPD), it reduces airway infection and can decrease exacerbations, therefore suitable for patients having chronic respiratory diseases particularly for diffuse pan bronchiolitis (DPB), a chronic non-infectious lung inflammation where extensive treatment of erythromycin is recommended.[7] Treatment with erythromycin has been reported to reduce the levels of interleukin-8 (IL-8) protein and the quantity of neutrophils in liquid taken from lungs from patients experiencing DPB and cystic fibrosis.[8]

Erythromycin at the dosage of 100 mg/kg body weight, stores in few major organs i.e. heart, liver, kidney and many other organs. Furthermore, patients on long treatment of antibiotics produce many reactive oxygen species (ROS). Likewise, erythromycin also produces reactive oxygen species which are mainly responsible of its hepatotoxic effect.[9]

Azadirachta indica typically known as neem is a tree which is famous for its therapeutic properties and has been utilized for quite a long time in medicine and agriculture. The Azadirachta is word derived from Persian language; the meaning of which is "Noble tree". It was discovered by a researcher named "De

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Jussieu" who provided the ordered nomenclature of it.[10] This investigation was particularly intended to evaluate the effect of Azadirachta indica on liver enzyme in albino wistar rats. Results were compared with the drug erythromycin that causes hepatic damage.

METHODOLOGY

This laboratory-based experimental study was carried out at the Anatomy Department of Baqai Medical University (BMU), Karachi and approval was taken from the Board of Advanced Studies and Research (BASR) and the Ethical Committee of University (ERC /IERB certificate no BMU-EC/2016-05).

Inclusion Criteria: Healthy Albino Wistar male rats having age of 13-14 weeks and weighing 180-200 grams were included in the study.

Exclusion Criteria: Mice with any obvious injury and disease were excluded. Rats died during the study were also excluded.

Eighty (80) healthy male rats were kept in the plastic cages (5 animals in each cage) under stern temperature (22 ± 2°C) and humidity (50-60%) in an interchanging 12-hours light/dark cycle. Rats were nourished with regular intake and water on a regular basis. Regulatory codes of National Institute of Health (NIH) were followed for control and testing on animals (National Research Council, 1996). Adaptation of animals for about 10 days was assured, preceding to the study.

Twenty (20) rats were divided randomly into four groups; "A", "B", "C" and "D". Group-A was kept as control and received no intervention and was fed with normal diet. Group-B only received erythromycin. Group-C animals received erythromycin 100mg/kg body weight and neem leaves extract in a dose of 500 mg/kg body weight orally. Group-D received only neem extract at the dose of 500 mg/kg body weight orally.

Erythromycin 500 mg tablets were used. Aqueous neem leave extract was prepared under the direction of Pakistan Council Scientific & Industiral Research (PCSIR), Karachi.

Group-A was the control group and received no intrusion and was nourished with normal diet. Group-B was given erythromycin 100 mg/kg body weight as a single dosage everyday by oral route for a period of 14 days feed via gastric lavage. Group-C received erythromycin 100 mg/kg body weight as a single dose plus aqueous Neem extract at the dose of 500 mg/kg body weight at once through gastric lavage for 14 days. Group-D was only given aqueous Neem extract at 500 mg/kg body weight of dose as a single dose by gastric lavage for 14 days.

No food was given to the rats overnight and the medication was given between 10-11 am for 14 days. Erythromycin and aqueous Neem leave extract were given with the help of gastric lavage.

Blood samples were taken through cardiac puncture in order to estimate the hepatic enzymes level such as ALP. Blood samples were taken with 5cc syringes into already marked tubes that contain antisera for analysis of hepatic enzymes using Biochemistry Analyzer Selectra E.

Data analysis was carried out by using Statistical Package for Social Sciences (SPSS) version 23.00. Data was comapred by using one-way analysis of variance (ANOVA) along with post-hoc tukeys test. The *p*-value of ≤0.05 was considered significant.

RESULTS

Eighty (80) grown-up Albino Wistar male rats of 13-14 weeks of age, weighted between 180 to 200 gms were studied. Twenty (20) rats were divided randomly into four equal groups. The ALP levels of group-B (Erythromycin treated animals) were highest. Mean value of serum ALP in group-A was 34.58 ± 2.89 IU/L, in group-B was 176.98 ± 10.53 IU/L, in group-C animals was 150.10 ± 2.28 IU/L, and in group-D animals was 35.33 ± 3.22 IU/L as shown in Table-I. The *p*-value was found to be statistically significant ($p<0.05$).

Table-I: Mean comparison of alkaline phosphatase enzyme (ALP) of the animals.

Parameters	Group- A Control, (n=20)	Group-B Erythromycin Treated (n=20)	Group-C Neem & Erythromycin treated (n=20)	Group D Neem treated (n=20)	*p*-value
Alkaline Phosphatase Enzyme (IU/L)	34.58 ± 2.89	176.98 ± 10.53	150.10 ± 2.28	35.33 ± 3.22	<0.05

While comparing serum ALP within different groups. There was noteworthy increase in ALP levels in group-B animals (176.98 ± 10.53 IU/L) vs group-A animals (34.58 ± 2.89 IU/L) with statistically significant result ($p<0.01$). ALP levels were raise in group-C (150.10 ± 2.28 IU/L) as compared to group-A animals (34.58 ± 2.89 IU/L) with the *p*-value <0.01. However,

ALP levels in group-C animals (150.10 ± 2.28 IU/L) in comparison to group-B animals (176.98 ± 10.53 IU/L) were found to be decreasing with the statistically significant result (p<0.01). There was significant decrease of ALP levels in group-D animals (35.33 ± 3.22 IU/L) were found when compared to group-B (176.98 ± 10.53 IU/L) and group-C (150.10 ± 2.28 IU/L) animals with the p-value found to be statistically significant (p<0.01) as shown in Table-II.

Table-II: Inter-group comparison of serum alkaline phosphatase enzyme (ALP) levels.

Groups	Inter-group Comparison	*p*-value
Group-A vs Group-B	Control vs. Treated	<0.01*
Group-A vs Group-C	Control vs. Protected	<0.01*
Group-A vs Group-D	Control vs. Positive Control	0.741
Group-B vs Group-C	Treated vs. Protected	<0.01*
Group-B vs Group-D	Treated vs. Positive Control	<0.01*
Group-C vs Group-D	Protected vs. Positive Control	<0.01*

DISCUSSION

In the present study, the drug erythromycin was given (100 mg/kg body weigh) to Albino wistar rats via oral route. Erythromycin with similar dose was used in a study conducted by Atul Rawat *et al*.[11] They studied the hepatotoxicity of liver on Albino wistar rats. Hepatic injury due to erythromycin was suggested to be due to the discharge of reactive oxygen species like superoxide anions and hydrogen peroxide. The degradation of hepatocyte cell (plasma membrane) and lipid peroxidation is due to these free radicals.[12] However, in another study, it was established that the drug erythromycin estolate, when given in a dose of 1500 mg/kg produces hepatotoxicity.[13]

Romeiro *et al*, in their research used erythromycin dose of 250 mg orally four times daily for the management of hepatic enchalopathy in patients of cirrhosis. They discovered that erythromycin can accomplish two evident targets in cirrhotic patients that is, it protects motility and transient time, thus decreases the overgrowth of the intestinal microbes. It also decreases the production of the ammonia in large and small intestine.[14]

Similarly Burr *et al*, in their research, used erythromycin for the prevention of severe conditions of COPD in the dose of about 200-500 mg/day orally in moderate to severe COPD patients with recurrent exacerbation caused by upper respiratory tract infections. Erythromycin decreases the levels of neutrophil counts in the sputum and neutrophil elastase.[15]

In a recent study, the serum level of one major enzyme i.e., ALP was measured, often used as hepatobiliary biomarker. Increased ALP level indicated hepatic damage.[16]

In present study it was found that in group-B animals (erythromycin treated group), serum alkaline phosphatase (ALP) level was noticeably raised when compared with animals of group-A (control group). This increase ALP level can be due to necrosis followed by degeneration of hepatocytes causing discharge of enzymes into the blood and also specifies increase permeability of cell membrane. This is in agreement with the observations established by -Zentella *et al*. They found the raised levels of ALP after hepatocellular damage in experimental rats after the treatment with drug erythromycin. The authors also documented raised levels of ALP in paracetamol and carbamazepine induced hepatotoxicity.[17] In contrast, Chukwudoruo *et al*, found reduced levels of ALP in cirrhotic patients, when erythromycin was given in the dose of 250 four times a day orally when compared with the cirrhotic patients which were not given erythromycin.[18]

In our study group-C (erythromycin and neem treated animals) showed significant reduction in the serum ALP levels as compared to group-B, who were given erythromycin only. This suggests that increase levels of enzyme ALP decreased when compared to group B (Erythromycin treated animals), but the enzyme level did not approach to the normal enzyme level. Neem leaves extract preserves cell membranes components from free radical oxidation (ROS) and consequently prevent the liver enzymes to release into the blood.[19,20] This is in agreement with the conclusion of Nwobodo *et al*. They also found that neem extract seems to reduce the chemically induced hepatic injury in experimental rats, they suggested decreased levels of ALP by the administration of neem extract in paracetamol induced hepatotoxicity.[20]

Present study also indicated noticeable rise in serum ALP levels in group-B (erythromycin treated animals). This result was in accordance to the findings of Al–Timimi *et al*. They found raised levels of ALP and AST. The enzyme AST is another marker of hepatic injury. Once hepatocytes are injured, both AST and ALP are discharged into blood in larger amounts.[21] Chao Tian *et al*, also reported the increase levels of enzymes (ALP and AST) in erythromycin treated rats and suggested that in the erythromycin toxicity, hepatocytes release ALT and ALP in blood.[22]

In our study, levels of ALP were decreased in rats treated with erythromycin and aqueous neem leaves extract simultaneously. This was in accordance to the conclusions drawn by Johnson *et al*. They observed reduce levels ALP in acetaminophen induced hepatotoxicity in Sprague Dawley male rats. As ALP levels are sensitive marker of necrotic lesions within the liver, the release into the blood is suggestive of severe damage to hepatocyte membranes during paracetamol toxicity. When administered with neem extract the markedly decrease in levels was because of antioxidant protecting capacity of Neem leaves which prevents the paracetamol induced hepatic damage by reducing reactive oxygen species (ROS), protein, lipid and DNA damage.[23]

Aamer *et al*, also observed that the Azadirachta indica exerted its hepatoprotective activity by acting as an antioxidant agent, deterring lipid peroxidation in paracetamol toxicity. There was a noteworthy decrease in the hepatic enzymes AST, ALP and ALT after co-administration of neem extract with paracetamol.[24]

Present study indicated protective effects of aqueous extract of Azadirachta indica (Neem) on the liver by decreasing the levels of ALP enzyme in erythromycin induced liver toxicity which could be due to its powerful antioxidant effect. Additional studies on large number of animals are needed to further validate the result.

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LIMITATIONS OF STUDY

There were few limitations in this study as female rats could have been included in the study. Monitoring weight of animal was a challenge; we tried to maintain the weight of animals.

CONCLUSION

Aqueous neem extract showed protective effect on erythromycin induced hepatic damage.

Conflict of Interest: None.

Authors' Contribution

NJ: Concieved the idea, IR: Helped in literature review search, statistical analysis, AR: Helped in sampling, NJ: Literature review, LA: Helped in writing article, NR: Helped in article writing.

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