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Evaluation of Pharmacodynamic Interaction Between Berberine and Antibacterial Drug (Azithromycin) in Albino Wistar Rats

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Abstract

Background: Evaluation of pharmacodynamic interaction between Berberine and antibacterial drug (Azithromycin) in Wistar rats.

Method: The study aims to evaluate the antimicrobial and antitubercular activities of a combination of berberine and azithromycin against microorganisms (E. coli, S. aureus) and Mycobacterium tuberculosis by using Alamar Blue Assay respectively. The compatibility and hepatoprotective activities of Berberine were also carried out against the azithromycin-induced hepatotoxicity rat model.

Result: The combination of berberine and azithromycin does not show any compatibility with a single-drug investigated by the Rf value, and synergistic effect against S. aureus and E. coli represented a significant zone of inhibition. Azithromycin showed in-vitro antitubercular activity at all concentrations, and berberine at 6.25µg/ml. Berberine and azithromycin co-administered for 28 days result in increased body weight, albumin level, total protein, and decreased serum transaminase, alkaline phosphatases, and total bilirubin significantly.

Conclusion: The combination of berberine and azithromycin does not show physical interaction, results in no alteration in the drug absorption, and showed antibacterial activity. Phytoconstituents showed antitubercular activity at different concentrations. The hepatoprotective action of berberine was proven by the evaluation of biomarkers of liver and body weight. Histopathological reports of the liver revealed that herbal mixture improves hepatocellular vacuolation and infiltration of inflammatory cells. Finally, based on in-vivo studies concluded that combination showed increased hepatoprotective activity in comparison to individual herbal drug.

Keywords Berberine, Azithromycin, Hepatoprotective, Antimicrobial, Zone of inhibition, Antitubercular activity.

Introduction

The variations in the drug effects due to present use of additional drug or drugs called it as drug-drug interaction (1).The wide-range of safety margin drugs should be used so that any unexpected interactions do not cause toxicity. Drug interactions should be measured as a promising reason of any unexpected complications (2). By various ways fundamental interactions can be categorized as pharmacodynamic or pharmacokinetic. As this research work majorly focused on pharmacodynamic interaction thateffects when there is any change in the expected or predicted pharmacological action of any drug due to its interference to a drug with a second drug at its target site. The significance of this interaction causes the additive effect, combined effect, or

opposite Effect (3), a strong CYP3A inducer rifampicin after co-administration of dasatinib, ACYP3A substrate results into significantly decreased (4)

According to World Health Organization, herbal medicines are defined as 'broad, labelled medicinal products that contain as active ingredients of any parts of plants (5). For the liver protection modern medicine has confined choices of drugs and therapies (6). In the field of herb drug interactions investigational information, patient reports and cases are rare or inadequate. The accurate incidence of drug interactions is significant but mysterious. Thus, herbal medicines comprise a mixture of pharmacologically active plant components that are stated to work synergistically to yield an effect larger than the sum of the effects of the single component. In Ayurveda, liver damage can be protected by using medicinal plants that is happened by various chemical substances and nutritive agents. Therefore, in recent years herbal drugs are recognized as safe, economical and have potential to cure several diseases and illness (7).

The major cause of infectious diseases is microbial infection. The treatment of infectious disease is negotiated by the expansion of antibiotic-resistantstrains of infectious pathogens (8).The major site of metabolism is liver and plays a crucial part to detoxify the countless of toxins consumed or formed during absorption of the food material (9-11). Though there are several drugs available that cause hepatotoxicity and among them majorly used mediators are antimicrobial drugs like antitubercular, macrolide antibiotics, antifungals etc. (12). The concomitant use of herbal medicines offers a perception to fight against antibiotic resistance (13).

The semisynthetic macrolide antibiotic that generally used in the treatment of various bacterial infections ranging from mild to moderate. It acts as bacteriostatic against several strains of Gram - positive bacteria like staphylococcus, meningitidis, streptococci (14). Azithromycin acts by binding to 50s ribosomal unit thus inhibited the protein synthesis of bacteria. It is used to treat several infections like acute liver injury, chronic bronchitis, sinusitis, disseminated mycobacterium avium infection etc. (15). Azithromycin usage are generally well-tolerated but including side-effects like dizziness, nausea, rashes etc. Azithromycin rarely causes hepatotoxicity and it resembles like other macrolide antibiotics likecholestatic hepatitis and because of its widely usage it causes drug induced liver injury also (16). The mechanism of liver injury of azithromycin is unknown but due its rapid onset described generally by hypersensitivity (17).

During this study, hepatoprotective effects of berberine against antibacterial drug was evaluated. Berberine is a natural isoquinoline protoberberine type of alkaloid that is extracted from several different species like *Berberine vulgaris, Berberine aquifolium* and traditionally used to treat several types of liver disorders (18). In past decades, afterextensive research designates that berberine having wide range of pharmacological activities like antimicrobial (19).' antifungal (20). immunoregulative (21). Berberine demonstrate the hepatoprotective activity through several studies including acetaminophen induced hepatotoxicity in mice (22), CCl4-induced hepatotoxicity in mice (18).The proposed mechanism of berberine expressed as when there is any toxic exposure happened to liver results into oxidative stress because of breakdown into free radicals which is highly reactive. As this oxidative phosphorylation activates tumor necrosis factor to liberate from stellate macrophagesand damaged liver cells that stimulates and express the several mediators and resulting tointroduce the series of inflammationin damaged hepatocytes. Down regulation of lipid peroxidation decreases in oxidative stress and improved antioxidant activity ensured by berberine (18). The present study reported the pharmacodynamic interaction between berberine and azithromycin.

Materials and Methods

Chemicals

Nutrient broth and agar from Sisco Research lab, Ethanol were procured from Research Labs Fine Chem. Industry, Azithromycin from SV enterprises, Silymarin from Research-lab fine chemicals. Total protein, Alkaline phosphatase (ALP), Total bilirubin, aspartate aminotransferase (AST) and Serum albumin were evaluated using kits of Pathoenzyme Diagonistics. All other chemicals which employed in this study were of analytical grade.

Procurement of phytoconstituent

Herbal industry was provided the herbal phytoconstituent Berberine as gift sample for research purpose.

In-vitro compatibility study

To carry out compatibility study of combination of berberine with azithromycin

Procedure: Solvent is pass on to the container and then draw a line from the base for spotting samples now dissolve the test sample & spot onto the line. After spotting, in the upward position put the plate inside the chamber and assure that the spots don't touch the distance of solvent. From the top of plate develop up to the 1cm and after that, bring out the plate from the chamber and vaporized the sample. Examine the plates by using Ultra Violet lamp to identify them and calculate the Retention factor (Rf) value by given method: -

$$\text{Retention factor} = \frac{\text{Distance from reference line travelled by solute}}{\text{Distance from reference linetravelled by solvent}}$$

In-vitro antimicrobial activity

To determine antimicrobial activity of combination of berberine with azithromycin by agar-well diffusion method against Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus)

Procedure: For growth of bacteria nutrient agar petri dish was prepared and by spread plate method the test culture was spread on plate. By using cork borer (sterilized with ethanol) wells were prepared in seeded plates. Standard drug (azithromycin) + Phytoconstituent (Berberine) was added in well & incubated. Pure solvent was used to prepare one well of control. Bacterial test plates were incubated at 32 to 37 ˚C for 48 hours. Around the well, test organism sensitivity to phytoconstituent & standard drug were indicated by clear zone of inhibition. Clear zone of inhibition was measured by its diameter (23).

In-vitro antitubercular activity

Assessment of in-vitro antitubercular activity against Mycobacterium tuberculosis by alamar blue assay (MABA).

Procedure: Anti-tubercular activity was evaluated against *M. tuberculosis* by using well recognized method MABA. In this method to reduce background fluorescence, black, clear-bottomed, 96-well microplate was used to perform antitubercular susceptibility test. Dimethyl sulfoxide (DMSO) was used to prepare initial drug dilutions, and 0.1 ml of 7H9GC media in the microplates were used to perform two-fold dilutions. 100 to 0.2 µg/ml of *Mycobacterium tuberculosis* in 7H9GC were added to each well of 96 well microtiter plate containing test compounds. The microtiter plates were incubated at 37°C of three prepared control. well plates containing drug and medium, bacteria and medium and medium only. Alamar Blue dye solution (20µl Alamar Blue solution and12.5 ml of 20% Tween 80) were mixed to day 7 of incubation to all the wells and plates were re-incubated at 37 °C. for 24 hours. Fluorometer was used to measure fluorescence and MIC was determined (24).

Experimental method

The Institutional Animal Ethics Committee (IEAC) was approved the experimental protocol (Protocol no. DYPIPSR/IAEC/17-18/P-24) formed as per norms of CPCSEA.

Evaluation of hepatoprotective activity of berberine in azithromycin induced hepatotoxicity

- Group 1: Rats received saline solution and rodent chowfor 28 days considered as normal control.

- Group 2: Induction of hepatotoxicity by using azithromycin for 28 days considered as diseased control.
- Group 3: Rats received silymarin and azithromycin for 28 days and considered as positive control.
- Group 4: Rats received berberine and azithromycin for 28 days.
- Group 5: Rats received berberine alone for 28 days.

Drugs were managed orally and intraperitoneally one time a day. Each animal food consumption was certain primarily and then weekly. On the basis of each cage food and water consumption was calculated.

On 28th day of the experimental period, the animals were anesthetized with ether and 1ml of blood was stored using retro-orbital method. Thebiochemical parameters were estimated when blood clots, centrifugedand serum was removed.After blood collections, animals were killed andexcision of liver and kidneys immediately and washed with phosphate buffer, saline and weighed up on electronic balance and transferred to 10% v/v formalin solution for fixation for 48 hours.

Statistical analysis

Results were expressed as mean ± SEM. By One Way ANOVA (analysis of variance) the statistical importance was designed followed by Bonferroni test using Graph pad prism-7 software. Thesignificant value expressed as p <0.05.

Results and discussion

In- vitro compatibility studies

Effect of outcome on compatibility studies of combination of berberine with azithromycin was shown in table 1. Rf value of combination of Berberine + Azithromycin was found to be 0.63 and 0.56 respectively which determine the comparison of Rf value of single drug with combination that does not shown any physical interaction with each other.

Table 1- Effect of treatment on compatibility studies of combination of berberine with Azithromycin

S.NO	DRUGS	Rf Value
1	Azithromycin (A)	0.64
2	Berberine (B)	0.56
3	Azithromycin + Berberine	0.63 (A), 0.54 (B)

In-vitro antibacterial activity

Effect of treatment on zone of inhibition of Berberine, Azithromycin and Azithromycin + Berberine against *S. aureus*was described in Figure 1

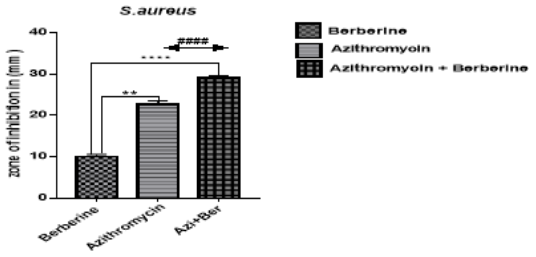

Figure 1. Effect of treatment on zone of inhibition against S. aureus

The values are represented by mean ± SEM (n=6 rats /group). ***P*< 0.01 compared with azithromycin; *****P* < 0.0001 compared with Azi + Ber; ####P< 0.0001 with compared with Azi + Ber by using One-way ANOVA (ONA)in respect to Bonferroni's test (BT).

Effect of treatment on zone of inhibition of berberine, azithromycin and azithromycin + berberine against *E. coli*

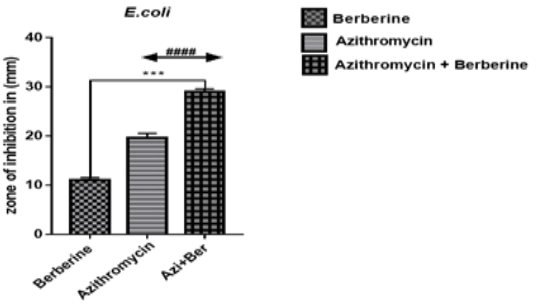

Figure 2. Effect of treatment on zone of inhibition against E. coli

The values are represented by mean ± SEM (n=6 rats/ group); ***P< 0.001, compared with azithromycin; ***P < 0.001 compared with Azi + Ber, and; ####P< 0.0001 compared with Azi + Ber by using ANOVA followed by BT.

In-vitro anti-tubercular study

Effect of treatment on antitubercular activity shown in table 2. of Phytoconstituents against *Mycobacterium tuberculosis* by Alamar Blue Assay (MABA) described in figure 3.

Figure 3. Alamar blue assay. Zero bacterial growth indicated by blue color while bacterial growth shown by pinkish color.

Table 2: MIC of different phytoconstituents (µg/ml)

Sample	100	50	25	12.5	6.25	3.12	1.6	0.8
T1 Azithromycin	S	S	S	S	S	S	S	S
T2 Berberine	S	S	S	S	R	R	R	R

NOTE: R-Resistant, S-Sensitive

Evaluation of hepatoprotective activity of berberine on hepatotoxicity induced by azithromycin

Effect of treatment on body weight

The body weight was acquired to be considerably decreased in group 2 as compared to group 1. On day 28 there were significant decrease in DC group (149 ±0.89) as compared to group 1 (168±0.69). The combination of Azithromycin and Berberine (169.5±1.04) showed significantly increased in body weight as contrastto negative and positive control group (168±0.68). The body weight was measured on 7, 14, 21, and 28th day shown in Figure 4.

Each value represents the mean ± SEM (n=6 rats/ group) by using ONA followed by BT. Group 2 is compared with Group1 Group (STD) 3, (Azh + Ber) 4 and (Ber) 5 are compared with (Azh)

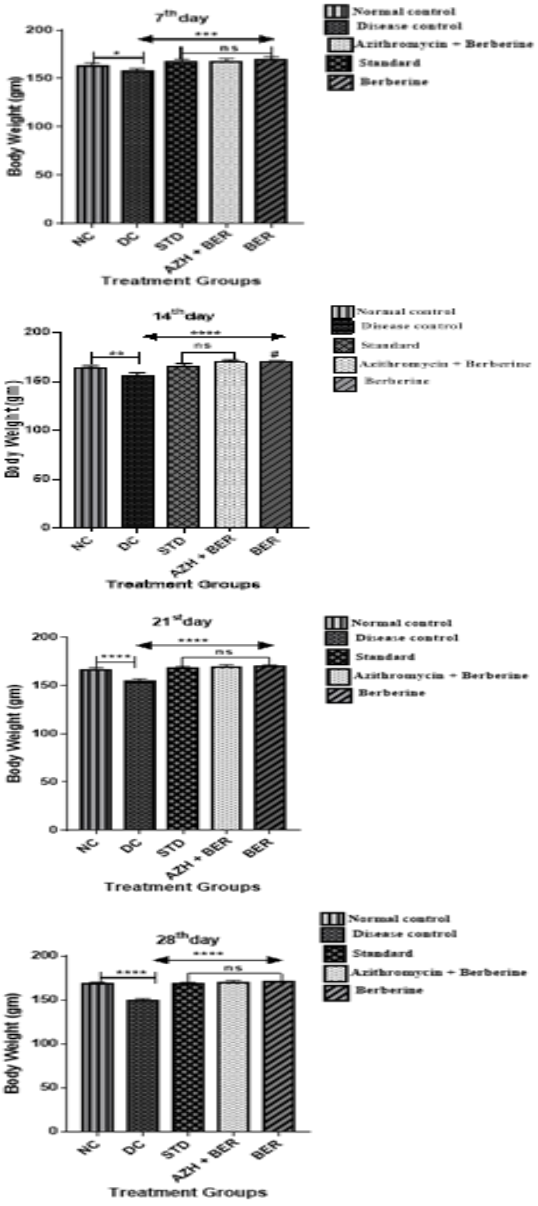

Figure 4. Effect of treatment on body weight

Each value represents the mean ± SEM(n=6 rats/ group) by using ONA followed by BT. Group2 is compared with Group1Group (STD) 3, (Azh + Ber) 4 and (Ber)5 are compared with (Azh)Group 2. Positive control group is compared with Group 4 and 5.The differences were measured statistically valid once the P< 0.05.

Effect of treatment on liver parameters

a. Effect of treatment on aspartate amino transferase (ast)

Effect of treatment on AST level was shown in Figure 5a. and shown to be considerably increased in group 2 (azithromycin- 95.59±2.33) as compared to normal control group (58.07±2.78) while test dose (74.93±1.35) showed significantlydecreased AST level as contrast to diseased and positive control group (66.04±2.11).

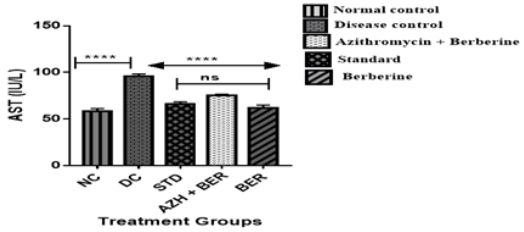

Figure 5a. Effect of treatment on AST

Each value represents the mean ± SEM (n=6 rats/group) by using ONA followed by BT. Group 2 is compared with Group 1 Group (STD) 3, (Azh + Ber) 4 and (Ber) 5 are compared with (Azh) Group 2. Positive control group is compared with Group 4 and 5. The differences were reflected statistically valid once the P< 0.05.

b. Effect of treatment on alanine amino transferase (ALT)

ALT level was defined to be substantially increased in group 2 (azithromycin- 40.10±1.18) as contrast to group 1(19.82±0.69) while test dose (26.91±0.83) showed significant decrease in ALT level as contrast to diseased and positive control group (22.54±0.61) that was described in figure 6b.

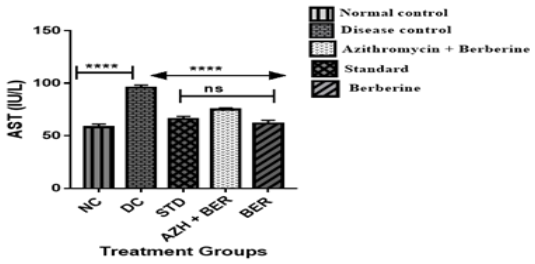

Figure 6b. Effect of treatment on (ALT)

Each value represents the mean ± SEM (n=6 rats/group) by using ONA followed by BT. Group 2 is compared with Group 1 Group (STD) 3, (Azh + Ber) 4 and (Ber) 5 are compared with (Azh) Group 2. Positive control group is compared with Group 4 and 5 The differences were reflected statistically valid once the P< 0.05.

c. Effect of treatment on Alkaline Phosphatase (ALP)

In figure 7c. ALP level was acquired to be considerably increased in group 2 (azithromycin-139.3±1.35) as compared to normal control group (73.21±5.95) while test dose (106.8±4.47) showed significantly decreased in ALP as contrast to diseased and positive control group (83.19±6.26)

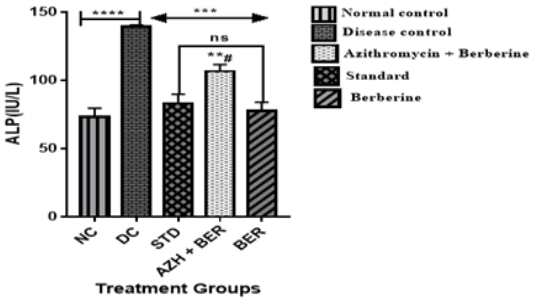

Figure 7c. Effect of treatment on ALP

Each value represents the mean ± SEM (n=6 rats/group) by using ONA followed by BT. Group 2 is compared with Group 1 Group (STD) 3, (Azh + Ber) 4 and (Ber) 5 are compared with (Azh) Group 2. Positive control group is compared with Group 4 and 5. The differences were reflected statistically valid once the P< 0.05.

d. Effect of treatment on total bilirubin

Total bilirubin was shown to be substantially increased in group 2 (azithromycin-0.77±0.03) as related to group 1 (0.28±0.01) while test dose (0.47±0.01) presented significantly decrease the bilirubin level as related to group 2 and group 3 (0.35±0.01) that was shown in figure 8d.

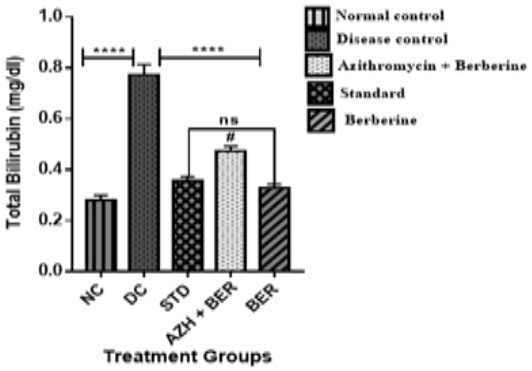

Figure 8d. Effect of treatment on total bilirubin

Each value represents the mean ± SEM (n=6 rats/group) by using ONA followed by BT. Group 2 is compared with Group 1Group (STD) 3, (Azh + Ber) 4 and (Ber) 5 are compared with (Azh) Group 2. Positive control group is compared with Group 4 and 5. The differences were reflected statistically valid once the P< 0.05.

e. Effect of treatment on total protein

In figure 9e. the significantly decreased in total protein as contrast to group 2 (azithromycin-4.38±0.14) as contrast to group 1 (6.91±0.16) while test dose (6.19±0.09) showed significant increase in level of total protein as compared to disease control.

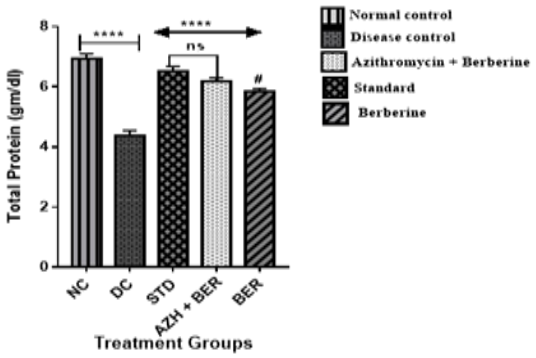

Figure 9e. Effect of treatment on total bilirubin

Each value represents the mean ± SEM (n=6 rats/group) by using ONA followed by BT. Group 2 is compared with Group 1 Group (STD) 3, (Azh + Ber) 4 and (Ber) 5 are compared with (Azh) Group 2. Positive control group is compared with Group 4 and 5. the differences were reflected statistically valid once the P< 0.05.

f. Effect of treatment on albumin

In figure 10f. the significantly decreased in albumin as contrast to group 2 (azithromycin-2.22±0.23) as compared to group 1 (4.38±0.09) while test dose (4.05±0.05) found to be significantly increasedthe level of albumin as compared to group 2.

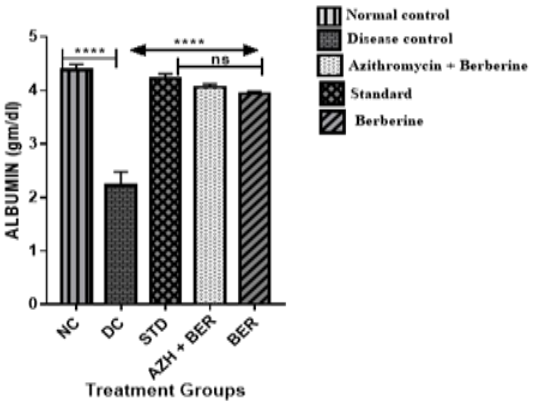

Figure 10f. Effect of treatment on albumin

Each value represents the mean ± SEM (n=6 rats/group) by using ONA followed by BT. Group 2 is compared with Group 1 Group (STD) 3, (Azh + Ber) 4 and (Ber) 5 are compared with (Azh) Group 2. Positive control group is compared with Group 4 and 5. The differences were reflected statistically valid once the P< 0.05.

Histopathological investigation

Microscopic examination of liver and kidney of normal group did not reveal any lesions of pathological significance. Microscopically, liver and kidney of negative control group rat fed with azithromycin revealed with multifocal hepatocellular vacuolation and multifocal infiltration of inflammatory cells respectively. Treatment of standard drug and test drug to rats did not revealed any pathological significance.

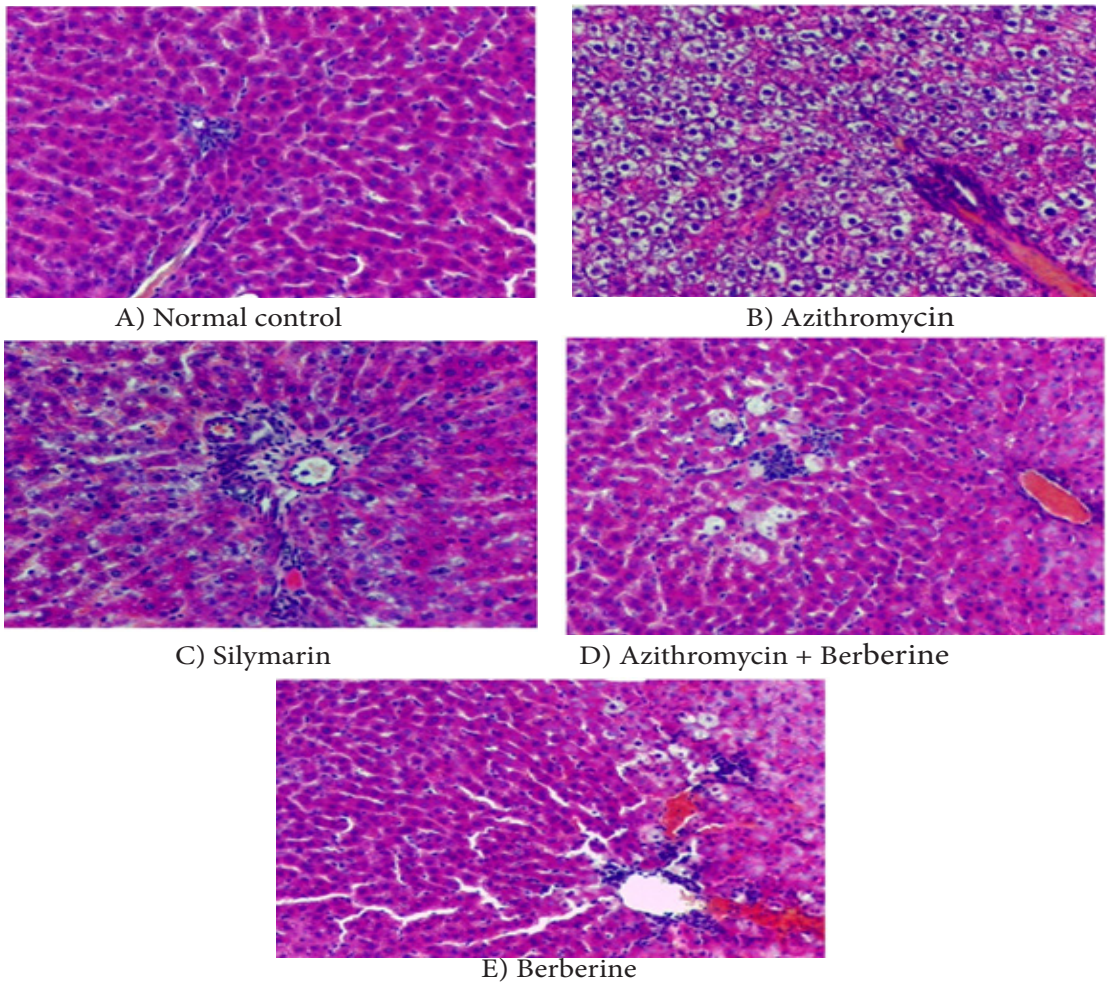

Figure 11. In histopathological examination of Liver observed- Normal hepatocytes, portal triad: In Normal control (A), normal Centrilobular hepatocytes: Silymarin (C), Berberine (E), normal periportal hepatocytes. Azithromycin + Berberine (D), treatment; and: Cholestasis with both hepatocellular and canalicular bile accumulation in Azithromycin (B) treatment.

Discussion:

Microbial infection is major cause of infectious diseases which can be led to morbidity and mortality. Though the antimicrobial agents are used to treat wide range of infections but they also cause various kind of toxicity. Irrational use of antibiotics leads to development of bacterial resistance. The most serious burden with antibiotic resistance is that some bacteria become resistant to nearly all of the readily available antibiotics. Therefore, this will be a challenge in future for treatment of resistant bacteria. One of the concepts to combat the emergence of antibiotic resistance is might be the use of herbal drugs concurrently. The wide use of herbal medicines in the society where population also uses prescription or synthetic medicines propose the adverse herb-drug interaction also. Potential herb-drug interactions can be prevented by prescribing them in such a manner that they do not developed any

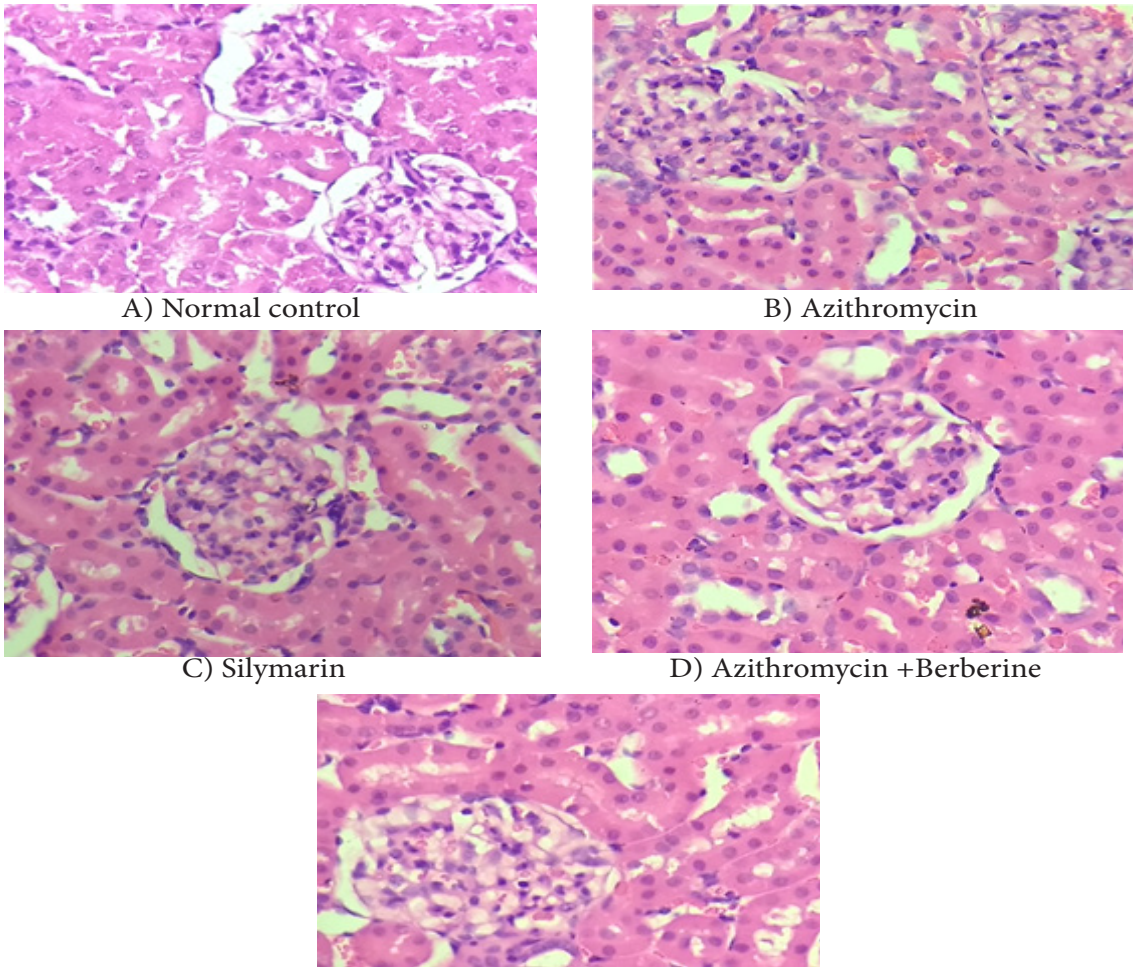

Figure 12. In histopathological examination of Kidney observed- Normal glomeruli and renal tubules: In Normal control (A), Silymarin (C), Azithromycin+ Berberine (D), Berberine (E) treatment; andmild Infiltration of Inflammatory cells and glomerular degeneration: Azithromycin (B) treatment.

untoward pharmacokinetic interaction, or by decreasing the dose in case both the herb and the pharmaceutical drug have the same therapeutic action.

In the present study, the pharmacodynamic interaction between Berberine and azithromycin was evaluated by using rat model. During this study, selection of berberine as an herbal drug because it shows hepatoprotective activity while macrolide antibiotic azithromycin has been chosen as hepatotoxic in nature.

In the present in-vitro study, to carry out the compatibility study of combination of Berberine with selected macrolide antibiotic with the help of TLC method by calculating the Rf value which shows that the Rf value of combination of Berberine + Azithromycin was found to be 0.54 and 0.63 respectively which conclude that when compare the Rf value of individual drug with combination it does not shown any physical

interaction with each other.

Varioussuggestion has demonstrated that the antimicrobial activity of berberine alone and in combination with ampicillin against a variety of bacterial pathogens like MRSA (Hyeon-Hee Yu et al, 2005) (25); Importantly, berberine having synergistic antimicrobial activityagainst *Candida albicans* (Y Liu et al,2017) (26)

But the present study shows the combination of Berberine with macrolide antibiotic azithromycin was studied to determine the antimicrobial activity against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) by observing the zone of inhibition and found that the combination showed potentiation in antimicrobial activity as compared with individual drugs.

During the present study, antitubercular activity was evaluatedof Phytoconstituents against *Mycobacterium tuberculosis* by Alamar Blue Assay (MABA) and berberine showed MIC at 6.25 µg/ml.

Recently in this study, hepatoprotective activity of berberine against hepatotoxicity induced by selected antibacterial drugs in Wistar rat was evaluated. Feng Y et al.2010, reported that the berberine holds hepatoprotective effects through foraging the peroxidative productsin contrast tocarbon tetrachloride persuadedliver toxicity which shows that serum levels of SGOT and SGPT were significantly decreased (27).

Domitrović R, et al 2011 demonstrated the protective effect of berberine against CCl4-intoxicated micewhich is associated withfree radical hunting, weakening of oxidative stress and inhibition of inflammatory response in the liver at dose 10mg/kg by i.p route.

In total the present study suggests that the combination of Berberine with isoniazid, rifampicin and azithromycin showed significant increase in body weight when compared with negative control. The rise in serum levels of serum glutamic-oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase (ALP) and total bilirubin inhepatotoxicity induced by antibacterial was markedly suppressed by combination of berberine with azithromycin. The decreased in total protein and albumin level were significantly increased by combination. From the histopathological reports of liver, it was concluded that the herbal mixture improves cholestasis with both hepatocellular and canalicular bile accumulation.

The herb drug interaction is to be studied seriously so as to increase effective and safe use of herbs and drugs together. The therapeutic potential of herbs can be wisely used to reduce the toxicity of these drugs.

Conclusion

The study selected was aimed at evaluation pharmacodynamic interaction between berberine and azithromycin in Wistar rats. The study was carried out with the objective of evaluation of *in-vitro* compatibility study, in-vitro antimicrobial activity and antitubercular activity with in-vivo hepatoprotective activity of Berberine against hepatotoxicity induced by selected antibacterial drugs. In-vitro compatibility study evaluated by Rf value of combination and showed no physical interaction, while evaluation of zone of inhibition of combination showed potentiation of antimicrobial activity and berberine showed antitubercular activity at specific MIC by MABA. Based on in-vivo studies concluded that combination showed increased hepatoprotective activity in comparison to individual herbal drug.

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Authors' contributions

Designing the study, data analysis and preparation of the manuscript was done by Mrs. Suchita Gupta and Reena Gupta, Dr.Jitendra Gupta. Authors read and approved the final manuscript.

Conflict of interests

The authors have no conflict of interests.

Ethical considerations

The study was approved by (Protocol no. DYPIPSR/IAEC/17-18/P-24) by the Institutional Animal Ethics Committee (IEAC) formed by as per norms of CPCSEA. Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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