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TOXICITY ASSESSMENT OF CLARITHROMYCIN IN DIABETIC WISTAR RATS TREATED WITH ROSIGLITAZONE

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ABSTRACT

Diabetes is a debilitating metabolic disorder characterized by chronically elevated blood glucose level above the normal range and affects mostly all kind of populations epidemiologically.

Objectives: In clinical situations, diabetic patients already on prescription of Rosiglitazone are prone to bacterial infection. To cure such infection Clarithromycin is prescribed particularly in Respiratory Tract Infections.

Materials and Methods: Diabetes was induced experimentally in overnight fasted Rats by intraperitoneal injection of Nicotinamide, 15 min prior to intraperitoneal injection of Streptozotocin (60 mg/kg). Nicotinamide (160 mg/kg) was freshly prepared by dissolving in distilled water and Streptozotocin was dissolved in freshly prepared 0.1M Citrate buffer. Since Rosiglitazone and Clarithromycin have found to exert minimal to mild toxicity in animals on these doses, i.e. 80 mg/kg and 400 mg/kg respectively, both these drugs were administered for 14 days to the animals to study the toxicity in diabetic condition. Parameters included for the study includes body weight changes, oral glucose tolerance test, biochemistry, hematology, urine analysis and histopathology.

Results: Clarithromycin aggravated hyperglycemia and reduced platelet count in diabetic condition. Further, combination of both Rosiglitazone and Clarithromycin reduced hepatotoxicity and nephrotoxicity in diabetic condition. Clarithromycin and Rosiglitazone combination showed markedly decreased TGL levels indicating Rosiglitazone's additive adverse effect in diabetic as well as non-diabetic condition.

Conclusion: Study inform about the two different class of drug which clinically can be used together. Though the combination has shown some promising effects on liver and renal functions in terms of Hepatotoxicity and Nephrotoxicity, concern about the reduced TGL and testicular toxicity cannot be over-looked and thus, further research is warned in this direction.

INTRODUCTION: Diabetes mellitus is one of the most rapidly growing diseases worldwide, and is a prime cause of excess cardiovascular morbidity and mortality in Western populations.

Other serious morbidities and mortalities are related to development of nephropathy (kidney damage), neuropathy (nerve damage), and retinopathy (blindness) due to diabetes.

Despite tremendous advances in medicine during the past century, there is still no cure, which means that effective prevention and treatment is of paramount importance to prevent future increases in disease burden [1, 2].

Various types of drugs are available in the market to treat type 2 Diabetes mellitus. Among them all, Thiazolidinedione class of drug are highly sensitive and usually prescribed by physicians. Thiazolidinediones (TZD) are known as insulin sensitizing agents since they work by improving the action of insulin, independently of the pancreas. Rosiglitazone and pioglitazone are the two agents currently available. Rosiglitazone is effective as monotherapy in the treatment of type 2 diabetes [3].

Rosiglitazone is generally well tolerated with the incidence of adverse effects similar to placebo. However, upper respiratory tract infections, injury, headache, back pain and hypoglycaemia are reported slightly more frequently than placebo in clinical trials [4]. To treat this upper respiratory tract infection, physicians usually prescribes macrolide like antibiotics [5].

Macrolides like Clarithromycin, Azithrimycin and Roxithromycin etc., are widely used drugs clinically. Diabetic patients, on treatment of Rosiglitazone prescribed by physician, if develops upper respiratory tract infection, which is being treated by macrolide likes antibiotics. Clarithromycin being easily available and having good potential to treat these infections have been selected for this study. Enough rodent data on toxicity of both these drugs i.e. Rosiglitazone and Clarithromycin, in diabetic condition is not available.

MATERIAL AND METHODS:

Chemicals: Streptozotocin (Lot-L25919) was obtained from Alexis Biochemicals, Mumbai. Nicotinamide (Batch no.-833544) was obtained from Sisco Research Laboratory (SRL), Mumbai. While both Rosiglitazone and Clarithromycin were the active pharmaceutical ingredients of Wockhardt Ltd, India.

Animals: The experimental protocol for this study was approved by an Institutional Animal Ethics Committee (IAEC), Wockhardt's Animal Ethics Committee under the guidelines of CPCSEA, Ministry of Environment, Government of India. Male Wistar rats (Wt. range-180-200 Gm) were obtained from the Animal House Facility, Wockhardt Research Centre, Aurangabad. Animals were housed in group of 6 in polypropylene cages with SS top grill containing autoclaved rice husk. All rats were provided with rodent pellet feed (Amrut Laboratory Animal Feed, supplied by Pranav Agro Industries, Pune, B.No: 560831) and water ad libitum and maintained in 12 hr light-dark cycle (Light: - 06:00-18:00. Dark: - 18:00-06:00), temperature 22±2^{o}C and relative humidity of 55±5%.

Induction of Diabetes: Diabetes was induced experimentally in overnight fasted Rats by intra-peritoneal injection of Nicotinamide (160 mg/kg), 15 min prior to intraperitoneal injection of Streptozotocin (60 mg/kg). Nicotinamide was freshly prepared by dissolving in distilled water and Streptozotocin was dissolved in freshly prepared 0.1M Citrate buffer immediately before use [6].

Experimental Design: 48 animals were weighed, randomized and divided into 8 groups (6 animals each, **Table 1**), and were given following treatment for 14 days by Oral route. Rosiglitazone (80 mg/kg) and Clarithromycin (400 mg/kg) both were suspended in 1% Tween 80, with the help of sonicator.

TABLE 1: GROUPING OF THE ANIMALS

Group	Animal No.	Treatment
I	1-6	Diabetic Control (DM Control)
II	7-12	Diabetic + Clarithromycin (DM + Clarithromycin)
III	13-18	Diabetic + Rosiglitazone (DM + Rosiglitazone)
IV	19-24	Diabetic + Clarithromycin+ Rosiglitazone (DM + Clari + Rosi)
V	25-30	Control
VI	31-36	Clarithromycin (Clari)
VII	37-42	Rosiglitazone (Rosi)
VIII	43-48	Clarithromycin + Rosiglitazone (Clari + Rosi)

Oral Glucose Tolerance Test: OGTT was performed on day 1 and 14 of the experiment. The rats were food deprived for 12 hr prior to the administration of an oral glucose load (2 g/kg body weight, 200 mg/ml). Blood samples were drawn by retro-orbital puncture method at 0, 30, 60, 90, and 120 min after glucose feeding. Collected blood samples were centrifuged at 3500±50 rpm for 15 min and serum was separated.

Glucose levels at each time point were determined using autoanalyser (Dade Behring-Dimension Xpand).

Hematological Examinations: Approximately 200µl blood was collected from each animal (n=4) by retro-orbital sinus in 0.5ml tubes (Tarsons Products Pvt. Ltd., Kolkata India) containing 20 µl 0.1% EDTA. The samples were kept on sample roller for 15-20 minutes at room temperature for uniform mixing of blood. Analysis was done by the auto analyzer (Beckman Coulter, AC.T). The parameters studied were WBC, RBC, Hb, HCT, MCV, MCH, MCHC, and Platelets.

Biochemistry Analysis: Approximately 1ml blood was collected from each animal (n=4) by retro-orbital sinus in 1.5ml tubes (Tarsons Products Pvt Ltd., Kolkata India). Blood was allowed to clot and was incubated for half an hour in incubator and centrifuged at 10000 rpm at temp. 18-22^{o}C for 10 minutes by Hermle-Z323K centrifuge machine. Biochemistry analysis was done by autoanalyser (Dade Behring-Dimension Xpand). The parameters studied were glucose, ALT, ALP, AST, BUN, CHOL, and CREA.

Urine Analysis: Approximately 200µl urine was collected from each animal (n=4) in 0.5ml tubes (Tarsons Products Pvt Ltd., Kolkata India). Analysis was done by the urine analyser (Yeongdong electronics co. Ltd). The parameters studied were Blood, BIL, UROB, KET, PRO, NIT, GLU, Ph, S.G, LEU.

Anti-oxidant Activity: Estimation of Lipid Peroxidation (LPO) [7], Reduced Glutathione (GSH) [8] in Erythrocytes was performed.

Histopathological Studies: The formalin fixed tissue pieces from liver, heart, kidney, lung, testis, and adrenals were serially dehydrated in alcohol and cleared in xylene and were embedded in paraffin blocks. The micro sections (4-5 microns thick) were cut and stained in hematoxylin and eosin (H&E) using standard method and examined for histopathological changes.

Statistical Analysis: Results are expressed as Mean ± S.E.M. and the data obtained were analyzed by 'Two way Anova followed by Bonferroni post test' and 'One-way Anova followed by Bonferroni post test'.

RESULTS:

Body Weight Changes: Body weights (gm) of all animals were measured at the regular interval of 3 days i.e., on days 1, 4, 7, 10 and 14. Mean % Body weight change of all the groups are shown in the figure 1.

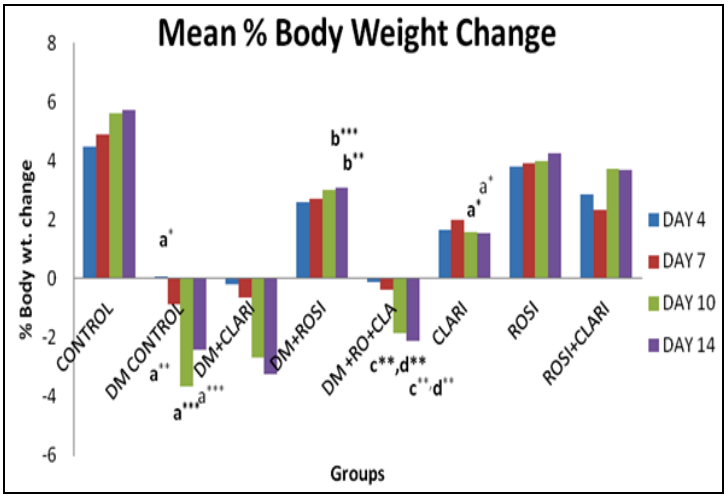


FIGURE 1: MEAN% BODY WEIGHT CHANGE

Results are expressed as Mean % body weight change, n=6, data analyzed by Two Way anova followed by Boneferroni post test. a^{*} = P<0.05 as compared to Control, a^{**} = P<0.01 as compare to Control, a^{***} =P<0.001 as compared to Control, b^{**} = P<0.01 as compared to Diabetic Control, b^{***} =P<0.001 as compared to Diabetic Control, c^{**}= P<0.01 as compared to Diabetic Rosiglitazone, d^{**}= P<0.01 as compared to Plain Rosiglitazone + Clarithromycin.

Oral Glucose Tolerance Test (OGTT): On day 1, OGTT was performed to check that whether diabetic animals have developed glucose intolerance or not. Nicotinamide-Streptozotocin treated group (blue line) showed significant amount of glucose intolerance while Control (brown line) showed no sign of glucose intolerance (**fig. 2**).

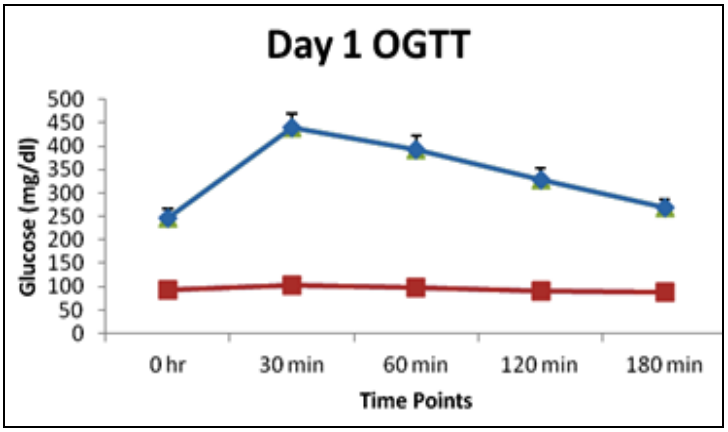


FIGURE 2: ORAL GLUCOSE TOLERANCE TEST FOR DAY 1.

Results expressed as Mean±S.E.M values, n=6

Haematology: Haematology was performed on day 14 using Beckman Coulter auto analyzer.

Platelet: Platelet count was significantly reduced in Diabetic + Clarithromycin and Diabetic+ Rosiglitazone + Clarithromycin treatment group. Note a reducing trend in Rosiglitazone+ Clarithromycin group as compared to concurrent control group (**fig. 3**).

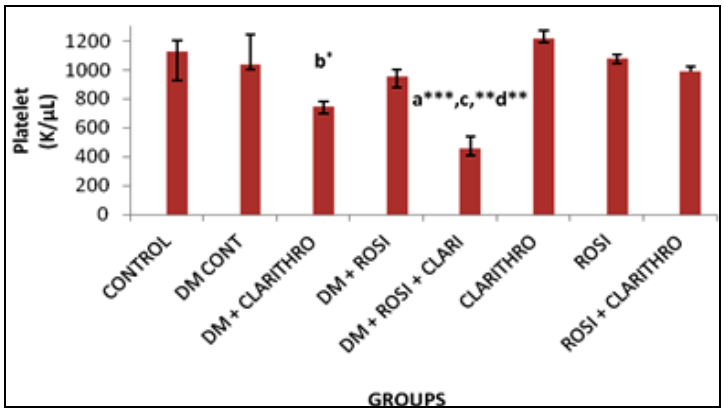


FIGURE 3: RESULTS EXPRESSED AS MEAN ± S.E.M, n=4

a*** = P< 0.001 as compared to DM + Control, b* = P< 0.05 as compared to Clarithromycin, c** = P< 0.01 as compared to DM+ Rosiglitazone, d** = P<0.01 as compared to Rosiglitazone + Clarithromycin.

Serum Biochemistry Analysis:

Blood Glucose: Serum glucose values remained high in all Diabetic groups compared to Control. Serum glucose levels were significantly increased in Diabetes with Clarithromycin treatment group. Rosiglitazone showing its glucose lowering potential by lowering glucose in diabetic animals, but not showing its activity of glycemic control with its full potential when used in combination with Clarithromycin. Mean ± S.E.M values are shown in **figure 4**.

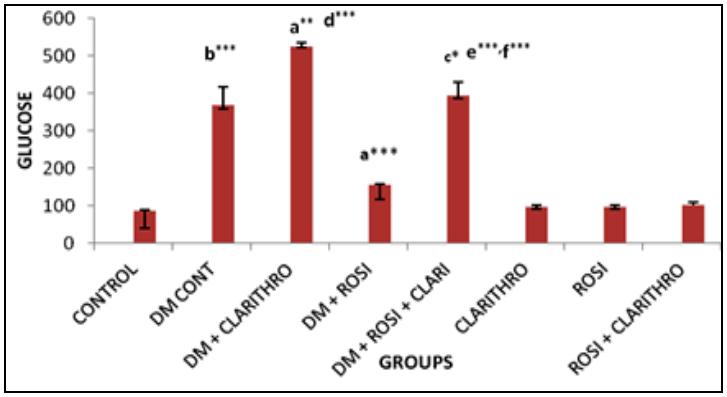


FIGURE 4: RESULTS EXPRESSED AS MEAN ± S.E.M VALUES N=4

Data are analyzed by One way Anova followed by Boneferroni post test. a** = P<0.01 when compared to Diabetic Control, a*** = P<0.001 when compared to Diabetic Control, b*** = P<0.001 when compared to Control, c* =P<0.05 when compared to DM + Clarithromycin, d*** = P<0.001 when compared to Clarithromycin, e*** = P<0.00 when compared to DM+ Rosiglitazone, f*** = P<0.001 when compared to Rosiglitazone+ Clarithromycin.

Alanine Aminotranseferase (ALT): In Diabetic+ Clarithromycin group, there were increased levels of ALT as compared to Clarithromycin group. There was an increasing trend in Diabetic + Clarithromycin group as compared to Diabetic Control, Diabetic Rosiglitazone and Diabetic + Rosiglitazone + Clarithromycin. Mean ± S.E.M values are shown in **figure 5**.

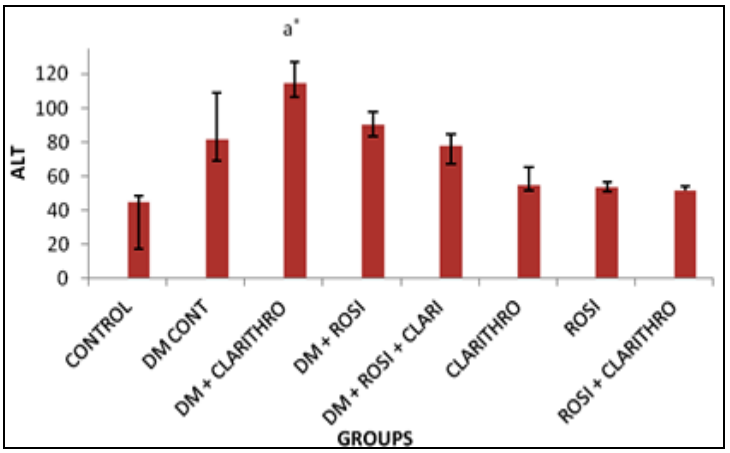


FIGURE 5: RESULTS EXPRESSED AS MEAN ± S.E.M VALUES, n=4.

Data are analyzed by One way Anova followed by Boneferroni post test. a*= P<0.05when compared to Clarithromycin.

Alkaline Phosphatase (ALP): There was no significant difference in ALP values in all the groups. There was just an increasing trend in Diabetic + Clarithromycin and Diabetic + Rosiglitazone + Clarithromycin group. Mean ± S.E.M values are shown in **figure 6**.

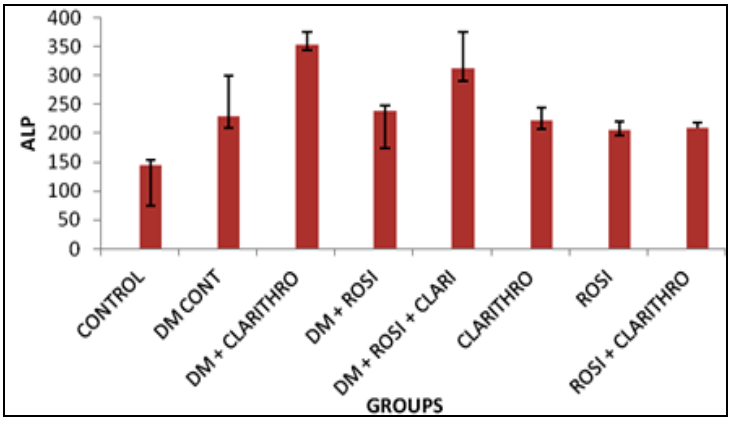


FIGURE 6: RESULTS EXPRESSED AS MEAN ± S.E.M, n=4.

Data are analyzed by One way Anova followed by Boneferroni post test.

Aspartate Aminotranseferase (AST): AST levels were significantly increased in Diabetic + Rosiglitazone as compared to concurrent Diabetic Control, Diabetic + Rosiglitazone + Clarithromycin and Plain Rosiglitazone (**fig. 7**).

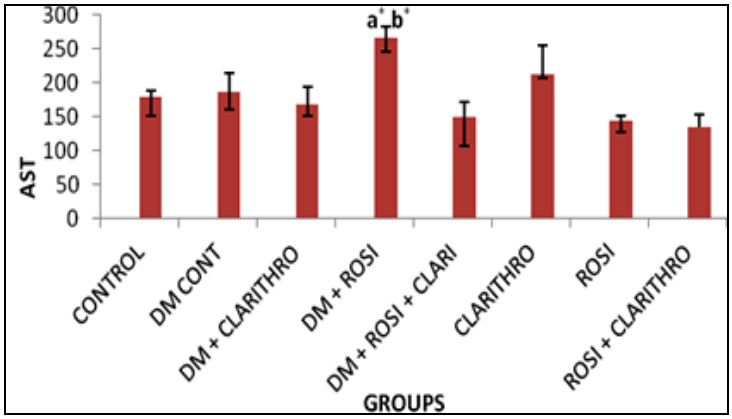


FIGURE 7: RESULTS EXPRESSED AS MEAN ± S.E.M, n=4

Data are analyzed by One way Anova followed by Boneferroni post test, a*= P<0.05 as compared to Diabetic + Rosiglitazone + Clarithromycin, b* = P<0.05 as compared to Rosiglitazone.

Blood Urea Nitrogen (BUN): BUN levels were significantly increased in Diabetic + Clarithromycin group as compared to Diabetic Control and Clarithromycin group. However, when given with Rosiglitazone, i.e., Diabetic + Rosiglitazone + Clarithromycin group, BUN levels were significantly reduced and were comparable to Diabetic Control groups (**fig. 8**).

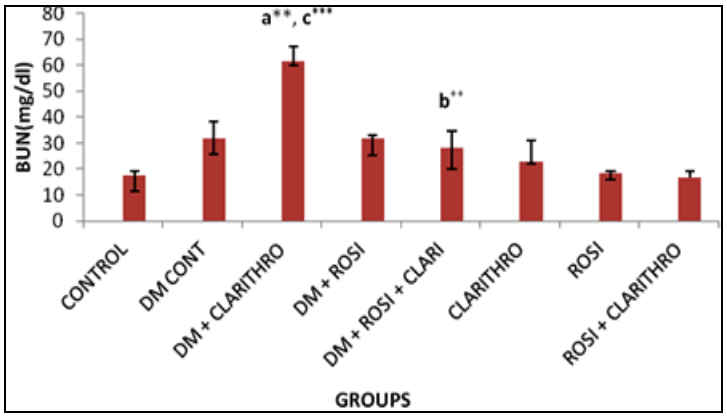


FIGURE 8: RESULTS EXPRESSED AS MEAN ± S.E.M, n=4

Data are analyzed by One way Anova followed by Boneferroni post test. a** = P<0.01 as compared to Diabetic Control, b** = P<0.01 as compared to Diabetic + Clarithromycin, c*** = P<0.001 as compared to Clarithromycin.

Triglycerides (TGL): In diabetic condition, Clarithromycin tend to marginally increase the TGL levels as compared to Diabetic Control. However when given in combination (i.e. Rosiglitazone + Clarithromycin) in both Diabetic and Non-Diabetic animals triglyceride level noticeably reduced. Rosiglitazone has lowered TGL levels in both, Diabetic and Non-Diabetic animals (**fig. 9**).

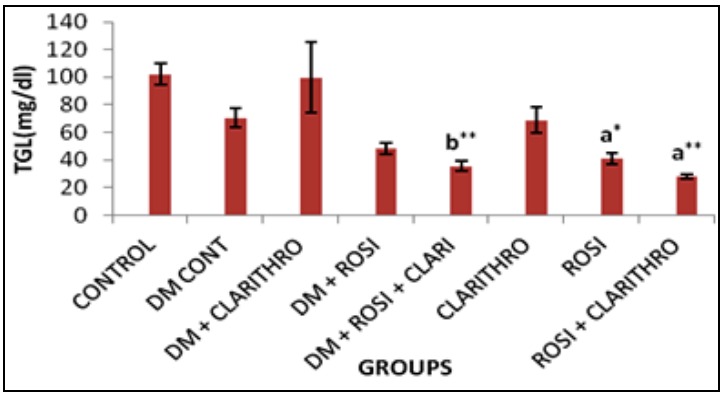


FIGURE 9: RESULTS EXPRESSED AS MEAN ± S.E.M, n=4

Data are analyzed by One way Anova followed by Boneferroni post test. a* = P<0.05 as compared to Control, a** = P<0.01 as compared to Control, b** = P<0.01 as compared to Diabetic + Clarithromycin

Urine analysis: No change in any of the parameters was observed in urine analysis except glucosuria. Glucose levels significantly increased in all the diabetic animals compared to non-diabetic animals. In diabetic condition, animals on Rosiglitazone treatment showed less glucose levels compared to other diabetic groups.

Anti-oxidant activity: Oxidative stress parameters like Lipid Peroxidation, and Glutathione Reductase were performed with standard method.

Lipid Peroxidation: Membrane damage indicated by increased Malondialdehide production was higher in all the groups including Diabetic Control when compared with Control (**fig. 10**).

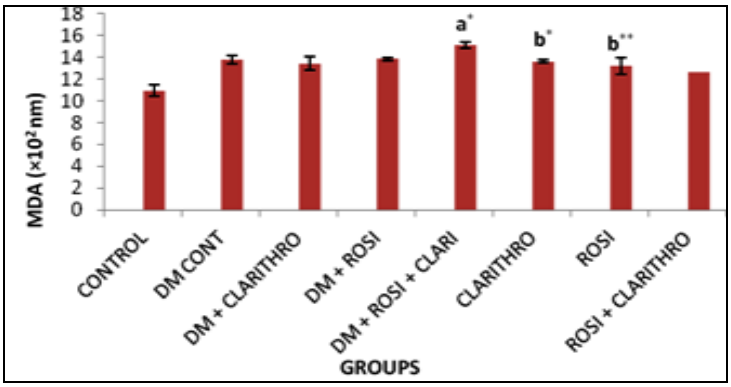


FIGURE 10: RESULTS EXPRESSED AS Mean ± S.E.M, n=5

Data were analyzed by One way Anova followed by Boneferroni post test. a* = P<0.05 as compared with Rosi + Clarithromycin, b* = P<0.01as compared with Control, b** = P<0.05 as compared with Control.

Glutathione Reductase: There was no significant difference in glutathione Reductase values in Diabetic groups and in its Concurrent control groups (**fig. 11**).

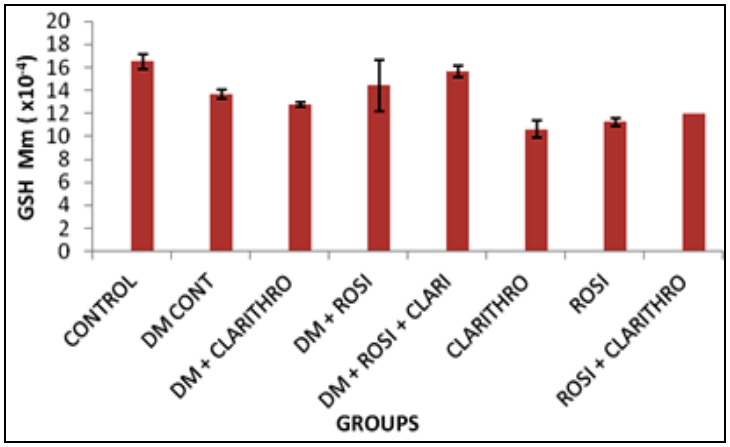


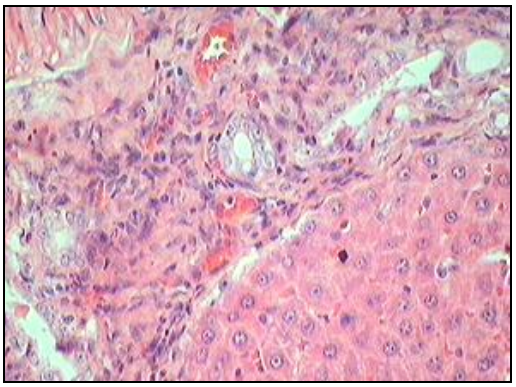
FIGURE 11: RESULTS EXPRESSED AS MEAN ± S.E.M, n=5

Data are analysed by One way Anova followed by Boneferroni post test.

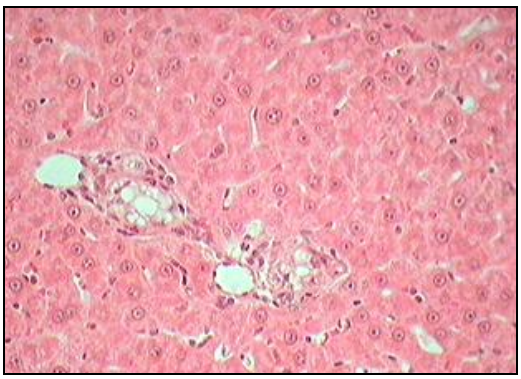
Histopathology: After collection of terminal blood collection animals were humanely sacrificed by using CO2 as euthanasia. Animals were exsanguinated by severing abdominal aorta and subjected to gross pathology observation by Veterinary Pathologist. Visceral organs like liver, kidneys, lungs, testes, adrenal glands, Heart, Brain were collected weighed and fixed in 10% Neutral buffered formalin fixative till processing for histopathology evaluation.

The formalin fixed tissue pieces from liver, heart, kidney, lung, testis, and adrenals were serially dehydrated in alcohol and cleared in xylene and were embedded in paraffin blocks. The micro sections (4-5 microns thick) were cut and stained in hematoxylin and eosin (H&E) using standard method and examined for histopathological changes.

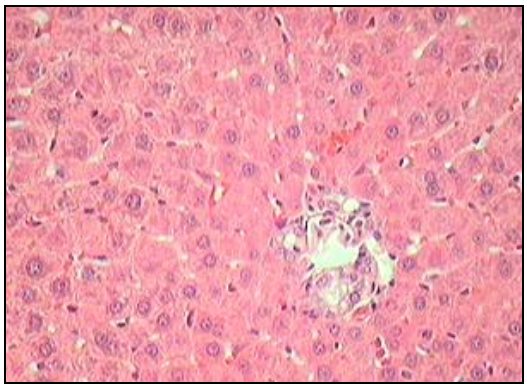
Liver, Kidney, Lungs and Testes organs were identified as target organs for Clarithromycin toxicity in diabetic and non-diabetic animals, whereas, for Rosiglitazone, liver and heart appeared to be target organs of toxicity on overall pathology evaluation during this study.



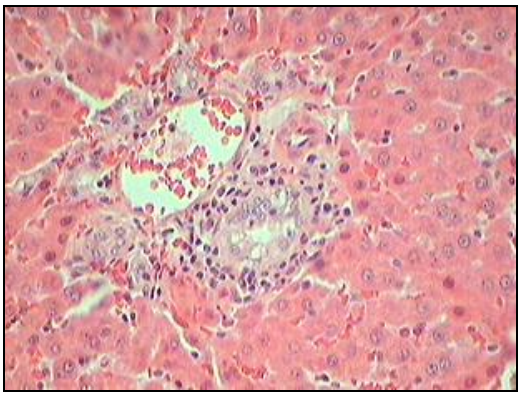
Liver from DM + Clarithromycin group showing Fibrosis in portal triad, bile duct proliferation & Vacuolation (40 X)



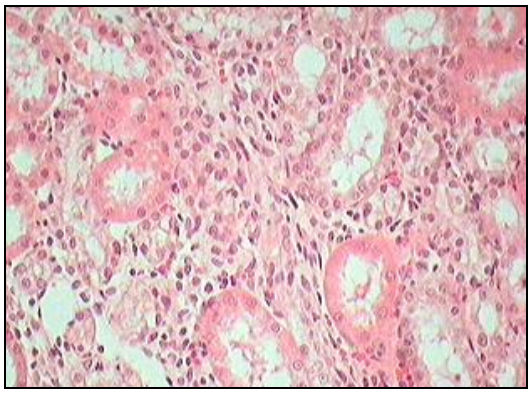
Liver from DM + Clarithromycin group showing Hepatocyte Necrosis & Vacuolated Bile ducts (40 X)



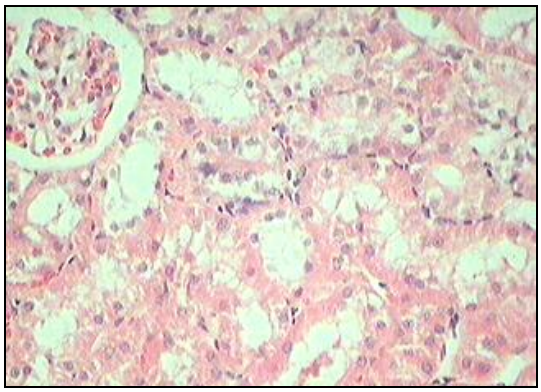
Liver from DM + Rosiglitazone group showing focal Hepatocellular Hypertrophy & Degeneration (40 X)



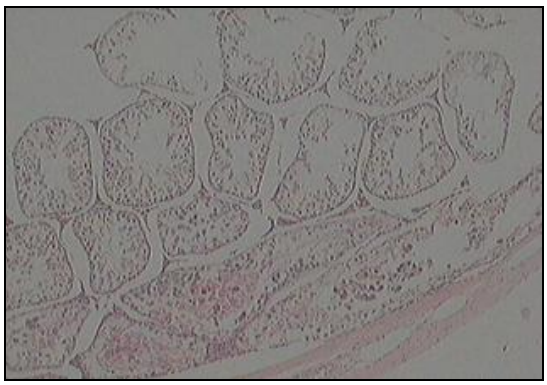
Liver from DM + Rosiglitazone + Clarithromycin group showing Bile duct proliferation & vacuolation; minimal portal triad fibrosis (40 X)



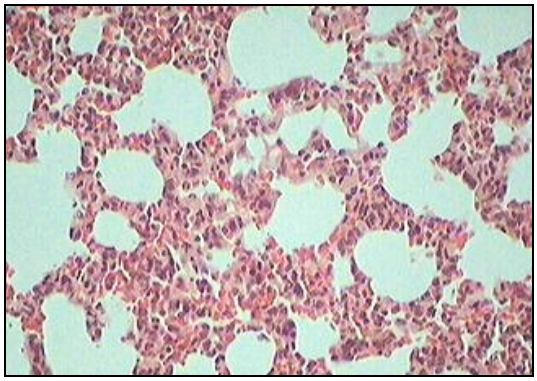
Kidney from DM + Clarithromycin group showing Tubular degeneration & interstitial fibrous tissue proliferation (40 X)



Kidney from DM + Rosiglitazone + Clarithromycin group showing mild degeneration & dilated tubules (40 X)



Testes from DM + Clarithromycin Group Showing degeneration & atrophy (10X)



Lung from DM + Rosiglitazone + Clarithromycin group showing minimally thick alveolar septa & Foamy alveolar macrophages (40 X)

DISCUSSION: Based on various studies, clinically it was concluded that Rosiglitazone was associated with a significant increase in the risk of myocardial infarction and with an increase in the risk of death from cardiovascular causes that had borderline significance [9]. There have been several conflicting reports regarding their effects on the liver that ranged from no toxicity to fatal acute liver failure [10].

Rosiglitazone therapy has been reported to increase respiratory tract infections [3], Macrolide and Ketolide being important antibiotics of choice for such infections, are commonly indicated for use in upper and lower respiratory infections and has a well established safety and efficacy profile. Macrolide antibiotics inhibit bacterial protein synthesis. Clarithromycin and Azithromycin are used to treat MAC and other nontuberculous mycobacteria.

Clarithromycin alters the metabolism of many other drugs that are metabolized by CYPs, leading to many potential drug interactions [11]. Clarithromycin induced hepatotoxicity is worldwide known [12]. Reports suggest that Clarithromycin is also associated with nephrotoxicity [13].

The present study has generated many interesting outcomes which are discussed here. The finding of decreased body weight in Clarithromycin, plain Rosiglitazone + Clarithromycin, DM + Clarithromycin, DM + Rosiglitazone + Clarithromycin was in conjunction with decreased food consumption in those groups (**Figure 1**).

It is well known fact that antibiotic drugs like Clarithromycin reduces the feed consumption through various mechanisms [14]. Similarly, increasing trend in body weight of plain Rosiglitazone treated group was due to increase feed consumption in that groups as well as adipogenesis like activity of Rosiglitazone [15].

Day 1 OGTT data (**Figure 2**) shows that animals treated with Nicotinamide - Streptozotocin showed marked glucose intolerance which indicated they developed type 2 diabetes mellitus [6]. While OGTT on day 14 indicated that Rosiglitazone showed its hypoglycemic activity in Diabetic + Rosiglitazone group, while in Diabetic + Clarithromycin group showed increased glucose levels compare to all other diabetic groups which indicates that Clarithromycin causes

hyperglycemia like situation in diabetic state. This observation was also seen in Diabetic + Rosiglitazone+ Clarithromycin group indicating Clarithromycin masking the inherent activity of Rosiglitazone to some extent with un-understood mechanism.

In Haematology, only platelet count was significantly reduced in Diabetic + Clarithromycin group while mild reduction was seen in Diabetic+ Rosiglitazone group, indicating that the drugs have potential to reduce platelets in DM [3, 16].

Significant platelet reduction was seen in combination, i.e., Diabetic + Rosiglitazone + Clarithromycin treatment group which indicates an additive adverse effect of both the drugs when used in combination on platelets. Even in non-diabetic animals this additive adverse effect of combination of the drugs on platelets was reflected (**Figure 3**).

In Biochemistry, levels of Glucose, ALT, AST, ALP, BUN and TGL were significantly altered. The decreased glucose levels than DM control in DM + Rosiglitazone group along with improved glucose tolerance & overall condition of animals, were indicative of classical anti-diabetic activity of Rosiglitazone [3].

Diabetic + Clarithromycin group showed increased glucose levels compare to all other diabetic groups which indicate that Clarithromycin might be causing hyperglycemia like situation in diabetic state. This observation was also seen in Diabetic + Rosiglitazone + Clarithromycin group indicating Clarithromycin masking the inherent activity of Rosiglitazone to some extent (**Figure 4**).

ALT and ALP levels were increased in Diabetic + Clarithromycin group which indicates Clarithromycin's hepatotoxicity [12]. Rosiglitazone also showed its minimal hepatotoxicity by increasing enzyme levels as compared to Diabetic Control in this study, but, when given in combination, i.e., DM + Rosiglitazone + Clarithromycin; hepatotoxicity was reduced and appeared comparable to Diabetic Control, serum biochemically.

This interesting observation indicates some sort of drug-drug interaction (but this time positively unlike adverse effect on glucose in combination (**Figure 5 and 6**). BUN levels were also significantly found to increase in Diabetic + Clarithromycin group as compared to Diabetic Control indicating Clarithromycin's nephrotoxicity [13].

However, when given in combination with Rosiglitazone, i.e., DM + Rosiglitazone + Clarithromycin the nephrotoxicity of Clarithromycin was brought to normal as compared to Diabetic Control, indicating combination to be better in the context of improving nephrotoxicity. The same trend was observed in non-diabetic animals also (**Figure 8**).

Triglyceride levels were markedly raised by Clarithromycin in Diabetic state. Rosiglitazone showed reduction in TGL levels that was as per the reports [17]. However, combination of both the drugs in diabetic state showed a significantly reduced TGL level which indicates Rosiglitazone's additive effect (**Figure 9**). Similar effect of Rosiglitazone was observed in non-diabetic treatment group too.

Urine analysis did not show any altered results. Rosiglitazone treated animal showed significantly less glucose levels as compared to other diabetic groups indicating its anti-diabetic activity. Membrane peroxidation was significantly increased in all treatment groups when compared with the Control group indicating the oxidative stress due to all treatments including DM control (**Figure 10**). GSH was significantly decreased in all treatment groups than the Control group indicating the retarded antioxidant machinery of body due to all treatments including DM control (**Figure 11**).

Histopathology data showed Minimal Hepatic Degeneration & focal Hepatocellular Hypertrophy in Diabetic Control animals which might be the effect of STZ induced free radical damage [18]. Plain Clarithromycin group showed bile duct proliferation & Vacuolation indicating phospholipidosis like changes.

Also Diabetic + Clarithromycin showed Fibrosis in portal triad, bile duct proliferation, & Vacuolation indicating phospholipidosis like changes, Hepatocyte Necrosis representing marked hepatotoxicity [12]. Also these results indicated increased risk of hepatotoxicity in DM animals than normal animals due to Clarithromycin.

Liver from Diabetic + Rosiglitazone and Plain Rosiglitazone group showed focal Hepatocellular Hypertrophy and Degeneration indicating minimal Hepatotoxicity due to increased peroxisome proliferation [10]. DM + Clarithromycin group showed tubular swelling, degeneration and interstitial fibrous tissue proliferation showing nephrotoxicity **[13]**. While DM + Rosiglitazone group showed negligible potential of nephrotoxicity. The reduced Histopathological observations in Kidney from DM + Rosiglitazone + Clarithromycin group indicated that combination improved nephrotoxicity of Clarithromycin in diabetic condition.

Heart from Plain Rosiglitazone Group showed minimal cardiac hypertrophy, might be due to increased plasma volume expansion as indicated by minimal decrease in HCT in hematology [19]. Testes of two animals from DM + Clarithromycin Group Showed degeneration & atrophy, clearly indicating testicular toxicity potential of Clarithromycin in diabetic condition as compared to plain Clarithromycin group in normal animals.

Lung from Plain Clarithromycin and Plain Rosiglitazone + Clarithromycin as well as DM counterparts like DM + Clarithromycin & DM Rosiglitazone + Clarithromycin groups showed Marked Alveolar foamy macrophages indicating phospholipidosis in lung due to Clarithromycin [16].

Another interesting finding in DM animals was decreased incidences & severity of lung infections in DM + Clarithromycin and DM + Rosiglitazone + Clarithromycin groups which might be due to potent antimicrobial activity of Clarithromycin, was also seen in plain Rosiglitazone + Clarithromycin group, but further work in this direction is warned.

CONCLUSIONS: From the above study following conclusions can be drawn;

1. Clarithromycin (400mg/kg) when given alone aggravated hyperglycemia in diabetic condition and masked anti-diabetic activity of Rosiglitazone (80mg/kg) when given in combination in diabetic condition.

2. Clarithromycin and Rosiglitazone combination improved hepatotoxicity in diabetic condition.

3. Clarithromycin and Rosiglitazone combination improved nephrotoxicity in diabetic condition.

4. Clarithromycin and Rosiglitazone when given alone markedly reduced platelet count in diabetic condition as compared to healthy counterparts and when given in combination, additive adverse effects of both drugs were seen on Platelets.

5. Clarithromycin and Rosiglitazone combination showed markedly decreased TGL levels indicating rosiglitazone's additive adverse effect in diabetic as well as non-diabetic condition.

6. Clarithromycin caused testicular toxicity in diabetic condition.

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