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Evaluation of hepatoprotective effect of galangin in isoniazid-rifampicin induced hepato-cellular damage

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Abstract
Hepatoprotective activities of phyto-constituents are widely documented. Galangin's radical scavenging ability has been extensively researched. The current study sought to determine Galangin's ability to protect rats from liver harm sustained by isoniazid and rifampicin. Wistar rats were used in this experiment. Hepatocellular damage was produced by a 21-day course of isoniazid (100 mg/kg) and rifampicin (100 mg/kg). Galangin doses used were 50, 100, and 200 mg/kg body weight. At the completion of the research, blood was collected and testing methods were performed to determine the antioxidant level. Galangin treatment (50,100, and 200 mg/kg body weight) restored AST, ALT, and ALP levels.SOD and catalase levels were also restored as a result of the medication. TNF-A-, IL-1, IL-6, MDA, and nitric oxide release all reduced. The results show that Galangin has a considerable hepatoprotective effect. Galangin's hepatoprotective impact might be achieved via its scavenging free radicals and cytokine regulating properties.

Keywords: liver, toxicity, antitubercular, peroxidation, galangin

Introduction
The liver is the primary organ involved in metabolism, secretion, and waste disposal. It also plays an important role in preserving, incorporating, and controlling balance in the body and is implicated in the majority of biological mechanisms for development, disease defence, nutrition replenish, power production, and procreation [1]. Many experts think that the liver is linked to the development of all ailments or the failure of different parts of the body to function properly. These are some of the primary and main functions of the liver is to aid in the metabolism of digestible additives such as food products, feed additives, liquor, and also most medications [2].
Medications could really cause a range of liver damage, from mild impaired function like elevated serum aminotransferase interaction to severe organ failure like liver injury or intraperitoneal cholestasis [3]. Many biochemical markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), cholesterol, bilirubin, triglyceride, and gamma-glutamyl transferase, increase with liver damage, while albumin (ALB) synthesis and total protein (TP) decrease [4]. Despite medical advancements, there are few synthetic drugs available to treat disease symptoms. Nevertheless, it is asserted that many plants have beneficial action in the treatment of liver illnesses.
Natural plants and plant-derived concoctions have been utilised by humans since the beginning of time. Many studies have been conducted in recent years to investigate the medicinal potential of numerous secondary compounds found in plants. According to the evidence, numerous secondary metabolites, such as polyphenols and flavonoids, are subject to a range of therapeutic actions in individuals [5]. Flavonoids are one of the most prolific and extensive secondary metabolite groups, and they are immensely beneficial to humans not just for their addition to plant colour, but also for their numerous biologically members currently [5]. Protracted use of various dietary sources (high in phenolics) has been shown to be preventive against the onset and treatment of diabetes, malignancy, osteoarthritis, cardiac disease, and neurological illnesses, among other things [6-9].
Galangin, a non-toxic natural product, has been linked to a number of medicinal effects. A growing body of evidence suggests that galangin, a naturally occurring flavonoid, has anticancer properties through a variousmechanisms [10]. The phytocompound inhibits cell proliferation while also having antiangiogenic properties [11]. Furthermore, galangin has shown significant results in the treatment of various cancers when combined with some other phytoconstituents or gold nanoparticles [12]. Galangin has been shown in studies to not only improve pre-existing hepatic steatosis but to also prevent the formation of stenosis by trying to promoteGalangin hepatocyte autophagy [13]. The present work is aimed to determine the protective effect of Galanginas on Isoniazid-rifampicin induced Hepatocellular damage in rats.

Experimental
1. Animals
Wistar rats (180-200 g) of either sex were housed in polypropylene cages, maintained under standardized conditions (12 h light/dark cycles, 28±2°) were used in the study. Animals were provided with standard pellet

food and had free access to drinking water. All the animal study protocols were duly approved by the Institutional Animal Ethics Committee.

2. Chemicals
Galanginwas purchased from Central Drug House, Mumbai, India. All the other chemicals used in the study were of analytical grade.

3. Selection of dose
As per studies performed by [14] Sivakumar *et al*, Galangin was used in the dose of 50, 100 and 200mg/kg of body weight

4. Animal group and dosing
Animals were divided into six groups with six animals in each
Group I Normal Control
Group II Isoniazid (100mg/kg) + Rifampicin (100mg/kg)
Group III Isoniazid (100mg/kg) + Rifampicin (100mg/kg) + Silymarin (100 mg/kg)
Group IV Isoniazid (100mg/kg) + Rifampicin (100mg/kg) + Galangin (50mg/Kg)
Group VI Isoniazid (100mg/kg) + Rifampicin (100mg/kg) + Galangin (100mg/kg)
Group VI Isoniazid (100mg/kg) + Rifampicin (100mg/kg) + Galangin (200mg/kg)

On the 21st day that is, after 48 h of pharmacological treatments, blood was withdrawn by a retro-orbital puncture for the estimation of biochemical parameters. After that, animals were sacrificed under ether anaesthesia. The liver was collected, washed and used for histopathological studies.

5. Biochemical analysis
Blood samples were collected into the epindrop tubes and centrifuged for 10 min at 7000 rpm using micro-centrifuge to separate the serum. The levels of serum glutamic oxaloacetic transaminase (SGOT/AST), serum glutamic-pyruvic transaminase (SGPT/ALT) serum alkaline phosphatase (SALP) were estimated using commercial kits (Span Diagnostics, India).The determination of total bilirubin was performed according to the standard principles and procedures of the kit manufacturer manual (Tulip Diagnostics, India).

6. Antioxidant enzymes
6.1 Superoxide dismutase assay
Superoxide dismutase (SOD) activity in liver homogenate was determined [15]. The method was based on the generation of superoxide anions by pyrogallol autoxidation, detection of generated superoxide anions by nitro blue tetrazolium (NBT) formazan colour development and measurement of the amount of generated superoxide anions scavenged by SOD (the inhibitory level of formazan colour development). The liver homogenate was centrifuged to 10000 rpm for 15 minutes at 4ºC. To 0.25 ml of supernatant, 0.5 ml of triscacodylic buffer, 0.1 ml of 16% triton x- 100 and 0.25 ml NBT were added. The reaction was started by the addition of 0.01 ml diluted pyrogallol. Incubation was maintained for 5 minutes at 37ºC. The reaction was stopped by the addition of 0.3 ml of 2M formic acid. The formazan colour developed was determined spectrophotometrically at wavelength 430nm. Enzymatic activity was expressed as ug/gm of tissue.

6.2 Catalase activity
The catalase activity was measured according to the method of Sinha [16]. 0.1ml of liver homogenate was mixed with 1.0 ml of 0.01M phosphate buffer (pH 7.4) and incubated with 0.4 ml of 0.2M H_2O_2 at 37°C accurately for 1.0 min and the reaction was stopped with 2.0 ml of 5% potassium dichromate (1:3 with glacial acetic acid). Further, the samples were incubated in a boiling water bath for 15 min. Tubes were centrifuged at 5000 rpm for 15 min and the supernatant was used to quantify the amount of H_2O_2 to calculate catalase activity at 570 nm. One unit represents 1.0µmole of H_2O_2 consumed/min/mg protein.

7. Assay of serum cytokines
Serum levels of tumour necrosis factor- α (TNF-A-α) interleukin 1 beta (IL-1ββ) and Interleukin 6 (IL-6) were determined using specific ELISA kits (Krishgen, India) following the manufacturer's instructions. The concentrations of assayed cytokines were measured spectrophotometrically at 450 nm. Standard curves were constructed by using standard cytokines and concentrations of the unknown samples were determined from the standard plots.

8. Assay of oxidative stress and antioxidant defence system
The lipid peroxidation in the liver was assayed by measurement of malondialdehyde (MDA) formation [17]. The plasma concentrations of nitrate were determined based on the Griess reaction [18].

9. Statistical analysis

The results were expressed as mean ± SEM. Statistical analysis was carried out by using One way ANOVA followed by Dunnett's test and $p<0.05$, $p<0.01$, $p<0.001$ was considered significant.

Results

1. Effect of Galangin administration on marker enzyme levels and bilirubin

The administration of isoniazid and rifampicin to rats caused significant liver injury, as evidenced by increases in the amounts of liver-specific markers such as ALT, AST, and ALP. In the current investigation, there was still a significant restoration of antioxidant enzyme levels (AST, ALP, and ALT) in animals from groups IV to VI administered with Galangin (50, 100, and 200 mg/kg) (Figure 1-3). Pretreatment with silymarin also provided considerable preservation against isoniazid and rifampicin-induced liver failure. Bilirubin levels were raised in animals given Isoniazid-Rifampicin (Group II). The treatment with Galangin (Group IV-VI) showed a significant reduction in bilirubin peroxidation (Figure 4).

2. Effect of Galangin administration on superoxide dismutase and catalase activity

SOD levels were reduced in mice after they were given isoniazid and rifampicin. Nevertheless, as contrasted to the harmful control, Galangin (50, 100, and 200 mg/kg) generated a substantial ($p<0.001$) increase in SOD levels (Group II). In addition, Galangin (50, 100, and 200 mg/kg) supplementation in mice resulted in a significant (p0.001) increase in SOD and catalase values. Galangin supplementation resulted in decreased in SOD as well as catalase values (Figure 5, 6).

3. Effect of Galangin administration on cytokine levels

TNF-A-, IL-1, and IL-6 are important cytokines that are released throughout inflammation. The current investigation found higher levels of serum cytokines in rats given Isoniazid-Rifampicin (Figure 3b, 4a, 4b). TNF-A-α, IL-1β, and IL-6 levels returned to close to normal in Silymarin-treated mice (Group III). The administration of Galangin at doses of 50, 100, and 200 mg/kg (Group III, IV, and V) to rats resulted in a decrease in the levels of such cytokines. Group VI animals showed the most impressive outcomes (Figure 7-9).

4. Effect of Galangin administration on nitrate formation and lipid peroxidation

The characteristics of cellular stress include lipid peroxidation as well as nitrate production. A quantitative examination of the liver (for lipid peroxidation) and serum (for nitrate production) demonstrated that Isoniazid-Rifampicin combination resulted in enhanced biological distress (Group II). Galangin (Group IV-VI) administration resulted in a significant reduction in lipid peroxidation, as seen by reduced MDA generation. Isoniazid-Rifampicin therapy resulted in enhanced nitrate production (Group II). Galangin consumption (Figure 5b) significantly reduced increased nitrate levels as well as restored them to near-normal values (Group VI) (Figure 10, 11).

Discussion

Drug-related hepatotoxicity is among the most difficult difficulties doctors confront, since it is a significant cause of liver problems defined by structural as well as metabolic abnormalities in the liver, attributable to the liver's role as the primary drug detoxifying mechanism. Despite the evidence that it might induce liver failure, isoniazid is nevertheless used to treat Tuberculosis [19]. Earlier biochemical ideas characterized this sort of drug-induced liver damage asmetabolic idiosyncrasy,' that was assumed to be caused by the potentially lethal acetylhydrazine metabolite and not by an immune reaction. Current findings, meanwhile, supports an alternate explanation, namely that isoniazid is immediately bio activated to a hazardous intermediate in some individuals, resulting in an immunological reaction and liver harm [2]. The use of scavenging free radicals as well as antioxidants is one of the treatments for regulating and reversing liver injury. Antioxidants have been shown to reverse oxidative stress within the organism. The hepatoprotective efficacy of several antioxidants obtained from natural has been experimentally evaluated.

The purpose of this research is to see whether Galangin may cope with isoniazid-rifampicin-induced hepatocellular injury in rats [20]. Animals were given Galangin at test doses of 50, 100, and 200 mg/kg. Galangin supplementation resulted in antioxidant enzyme (AST, ALP, and ALT) recovery in animals from groups IV to VI. The treatment of Galangin (50, 100, and 200 mg/kg) to animals resulted in significant increases in SOD, catalase, and bilirubin levels. Lipid peroxidation was reduced to near-normal levels. Serum ALP and bilirubin levels are connected to the activity of liver cells.

Eukaryotes have evolved a robust defensive barrier that supports eukaryotic cells against damage caused by free radicals. Antioxidant enzymes such as SOD and catalase operate well to prevent free radical damage. Nonetheless, isoniazid and rifampicin affect liver activity by producing free radicals, which most likely engage with enzymes, creating disruption in their action and underlining hepatic damage [21]. Galangin therapy causes a considerable increase in these enzyme levels, highlighting their potential to remove reactive oxygen species. The increase in lipid-peroxide synthesis reveals the cellular injury. The increase in free radical generation protects cell structure and is linked to induced oxidative cellular damage.

IL-1 is a potent inducer of effector cells, particularly monocytes and neutrophils that exhibit the interleukin-1 receptor. Caspase-1 is activated when IL-1 is activated via the proteolytic cleavage route [22]. Regardless of the intracellular mechanisms involved, activation of inflammasomes results in caspase-1 recruitment and the

degradation of pro-IL-1b to IL-1b. The latter binds with the IL-1R and increases liver ion. Potential involvement in reducing aggressive redistribution of IL-1R-expressing cell to the liver, which is characterized by tissue inflammation [23]. The treatment of Galangin to experimental animals resulted in a decrease in IL-1, indicating that it is effective.

IL-6 is a potential mediator with multiple functions in the body. In the liver, IL-6 is a crucial stimulator of the acute phase of inflammatory response and infection prevention [24]. IL-6 is also a critical regulator of hepatocytes and a potent stimulator of hepatocytes. Continued relevance of the IL-6 signalling pathway, on the other hand, is damaging to hepatic tissues and may lead to the development of liver malignancies [25]. The combination of isoniazid and rifampicin causes a hepatic inflammatory response by raising the levels of primary responders like IL-6 and later responders like ROS. IL-6 is a pro-inflammatory cytokine which increases neutrophil inflow, prostaglandin synthesis, and B and T-lymphocyte involvement. In the current investigation, Galangin decreased increased levels of IL-6, demonstrating its ability to inhibit inflammatory responses.

Elevated ROS levels harm cells in a number of different ways, such as the oxidation of macromolecules like lipids, peptides, and DNA. Enhanced lipid peroxidation produced by ROS formation results in the loss of function and integrity of cellular membrane, which leads to a rise in quasi responsiveness to ions, disrupting membrane integrity as well as cell processes [26]. The delivery of Galangin resulted in a reduction in the concentrations of MDA, a biomarker of lipid peroxidation, in the current investigation. It is critical to highlight that Galangin is both an antioxidant and an anti-inflammatory agent [27]. As a result, it is possible that Galangin's antioxidant function is related to its hepatoprotective impact [28].

Nitride oxide is constantly created throughout the body, altering cellular metabolic regulation. Excess NO• production can cause a range of diseases, including irritation, malignancy, and some others [29]. NO• is reactive in aerobic conditions and generally interacts with oxygen to generate stable products such as nitrate and nitrite by forming intermediaries. These free radicals modify the functional activity of biological components [30]. Galangin may help to limit the formation of intermediate intermediates, so protecting cellular organelles. The NO• scavenging capacity of Galangin was demonstrated in a dosage-dependent fashion in the current investigation.

The liver is a critical metabolic part of the human body. Inside the liver, several essential activities such as calorie-genesis, cleansing, enzyme manufacturing, and drug biotransformation take place. The hepatic injury is associated with negative alterations that may lead to the pathophysiology of cirrhosis. The current findings support the beneficial impact of Galangin treatment in animal experiments.

Conclusion

The current study's findings highlight the protective impact of Galangin in reducing the adverse impact of isoniazid-rifampicin on rats. These encouraging findings might contribute to the formation of hepatoprotective bioactives.

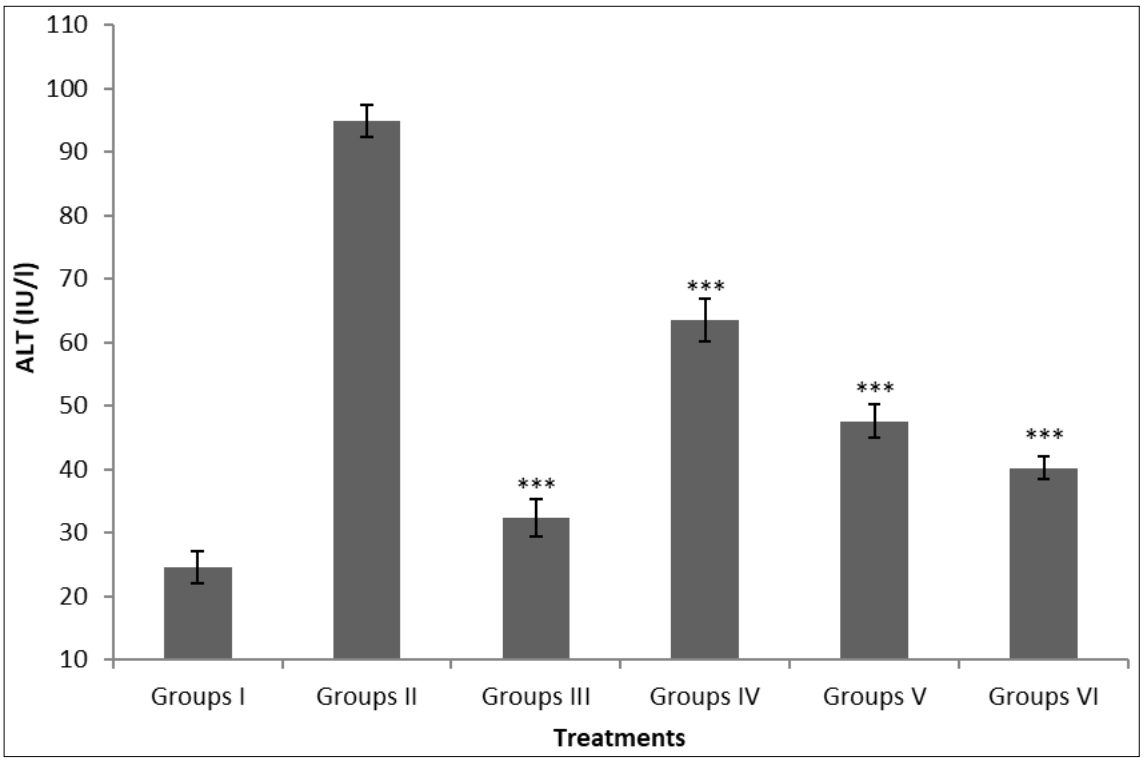

Fig 1: Effect of ascorbic acid administration on ALT levels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group.

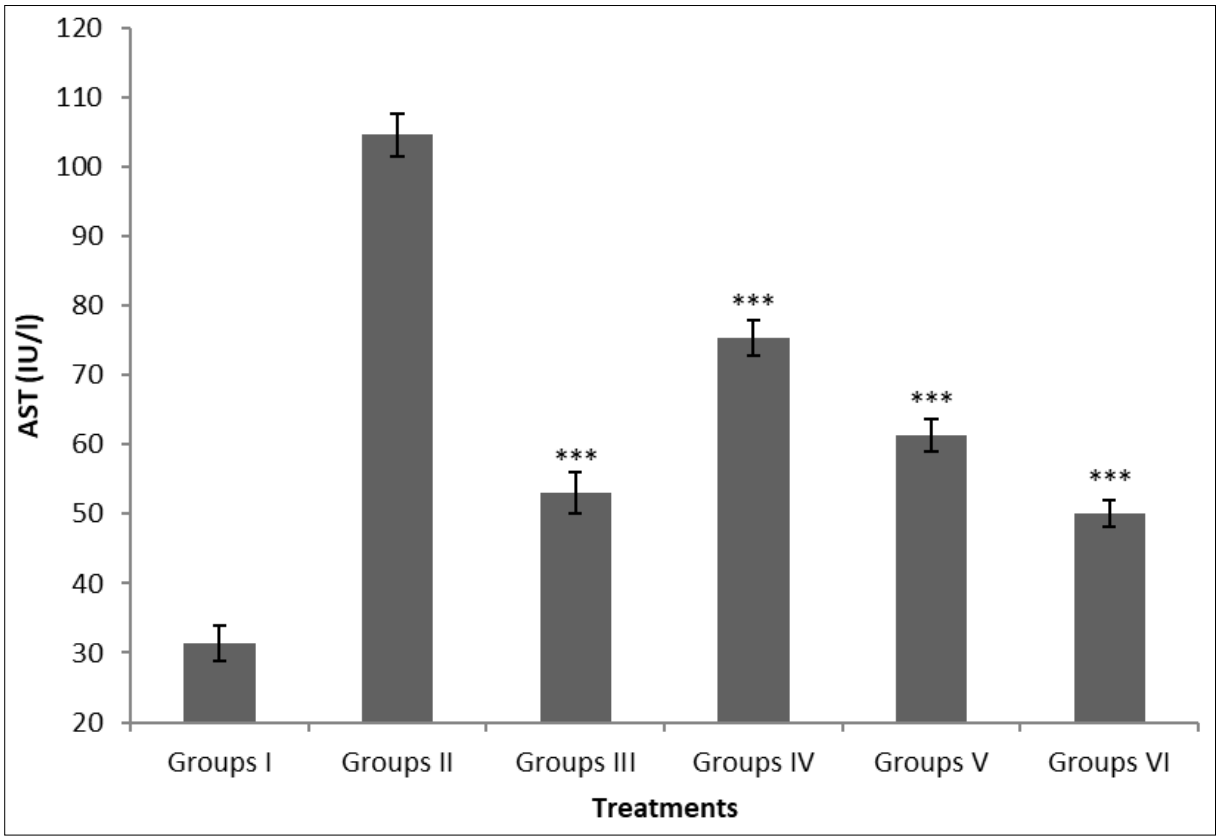

Fig 2: Effect of ascorbic acid administration on ASTlevels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group.

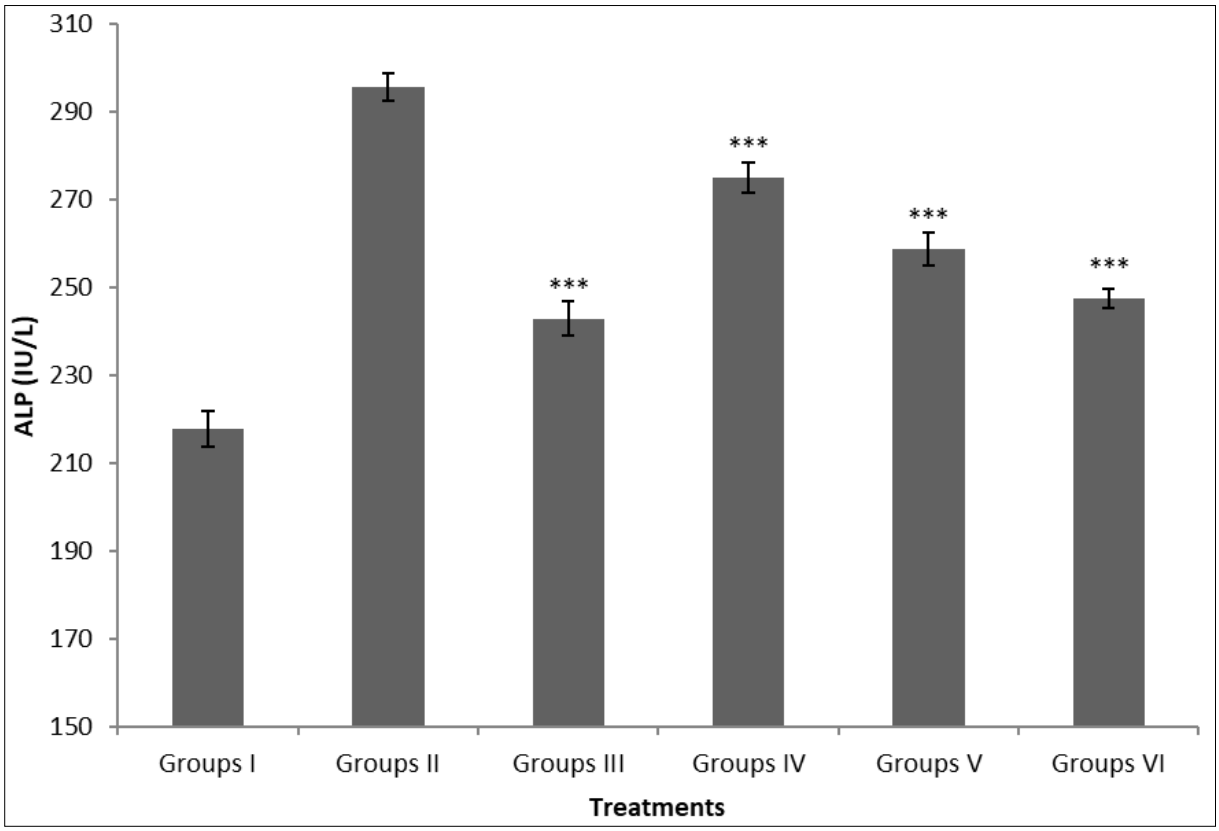

Fig 3: Effect of ascorbic acid administration on ALP levels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group.

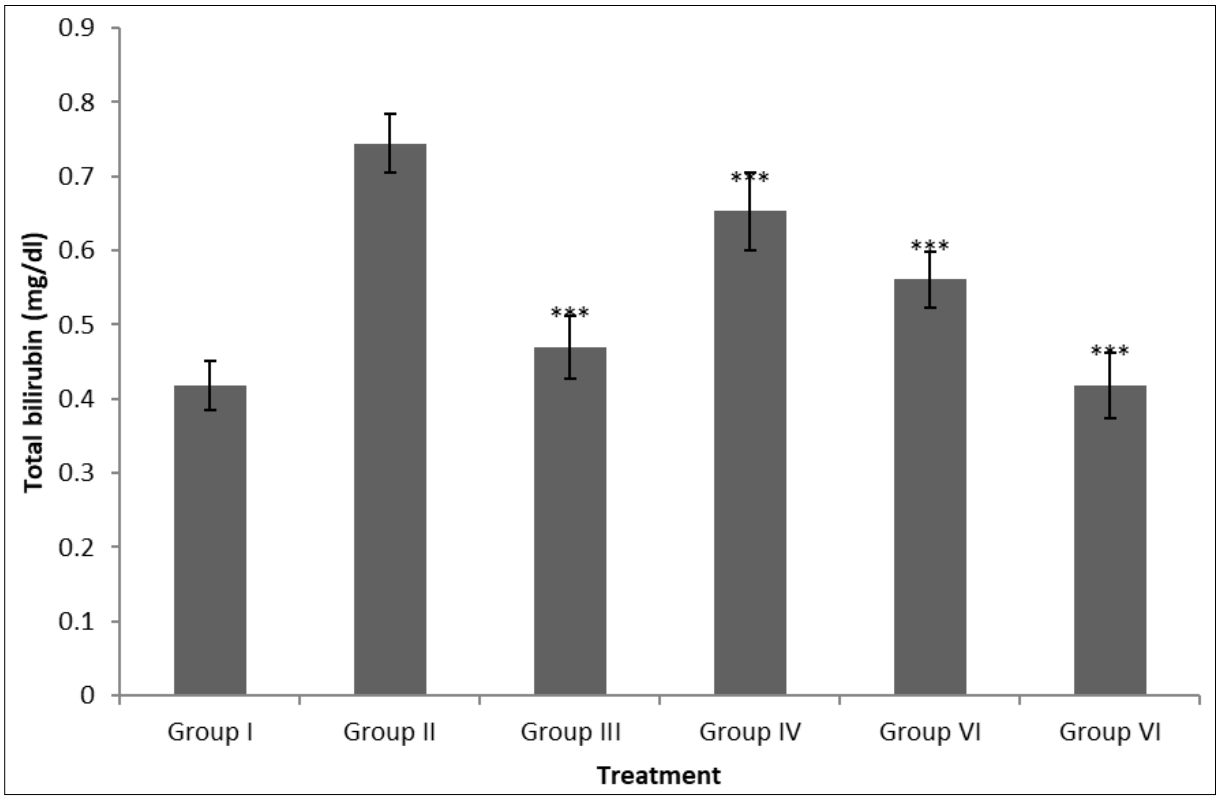

Fig 4: Effect of ascorbic acid administration on total bilirubinlevels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group.

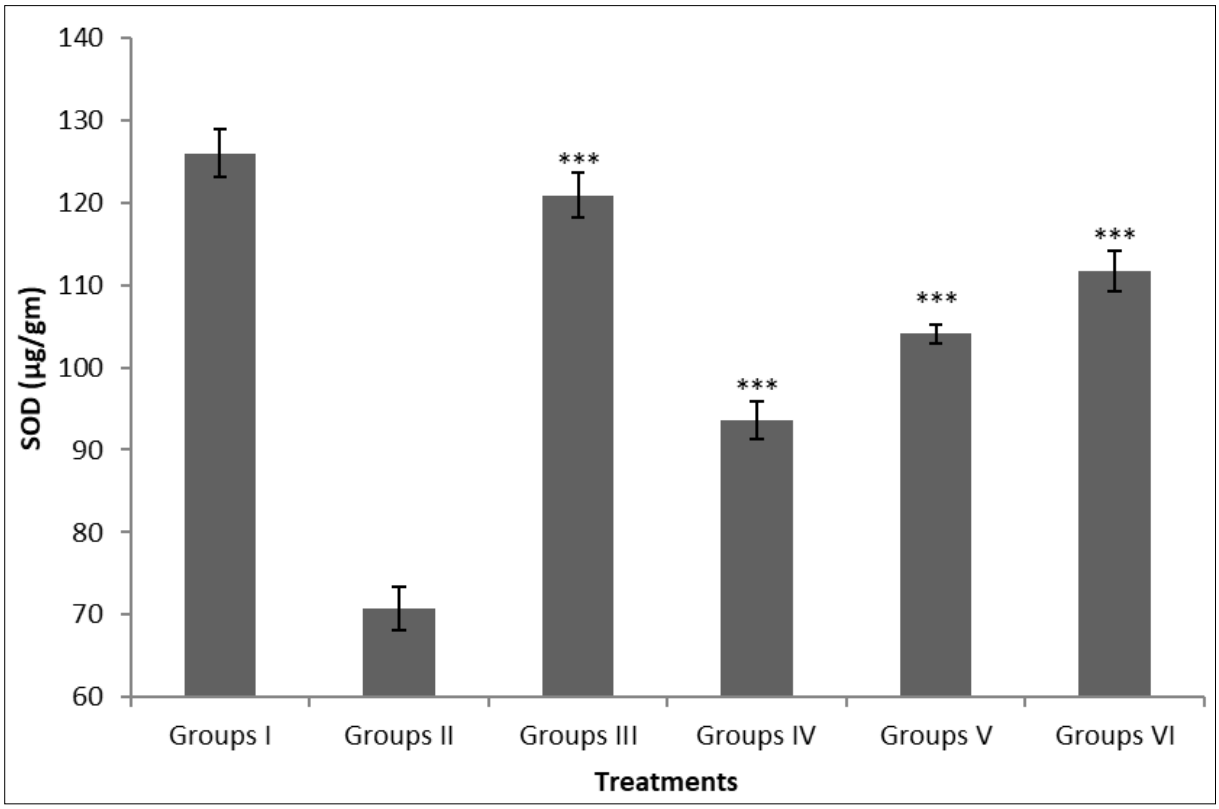

Fig 5: Effect of ascorbic acid administration on SOD levels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group.

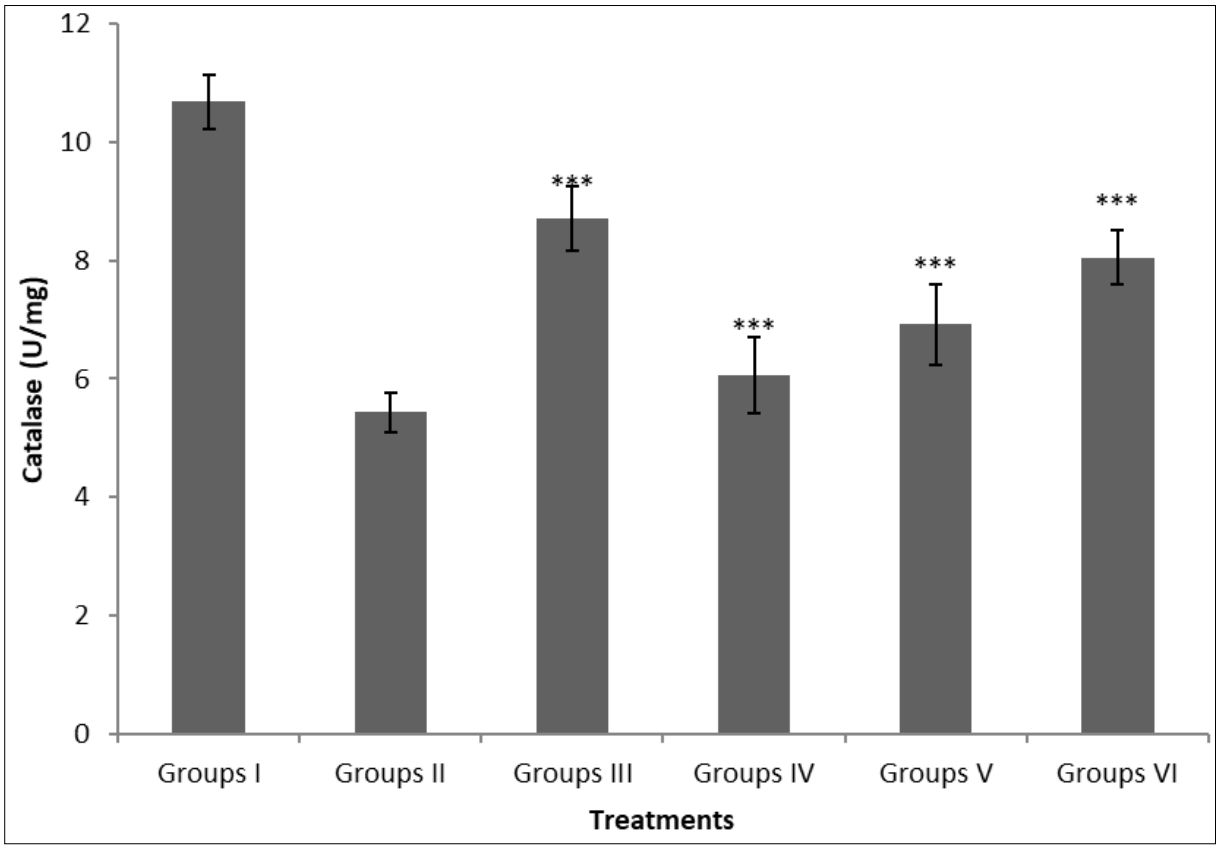

Fig 6: Effect of ascorbic acid administration on catalase levels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group.

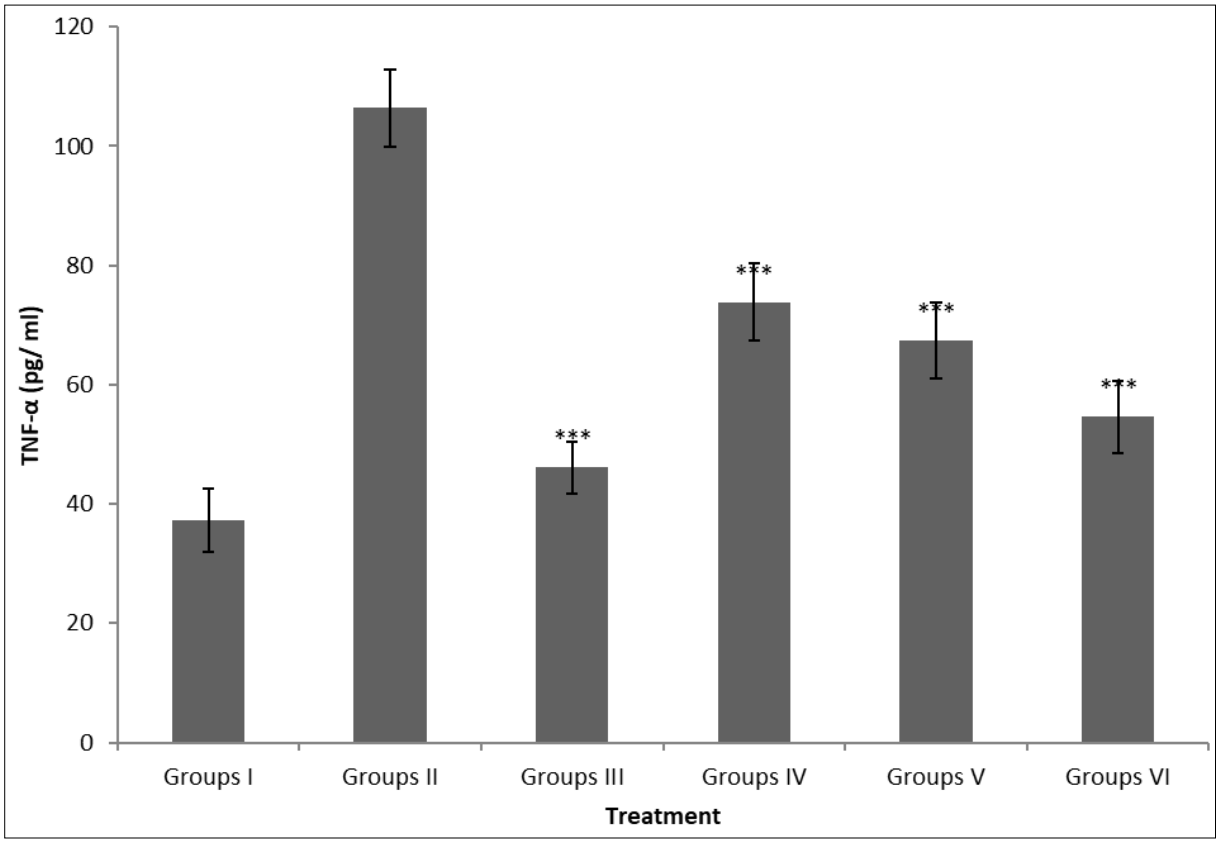

Fig 7: Effect of ascorbic acid administration on TNF-A-α levels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group.

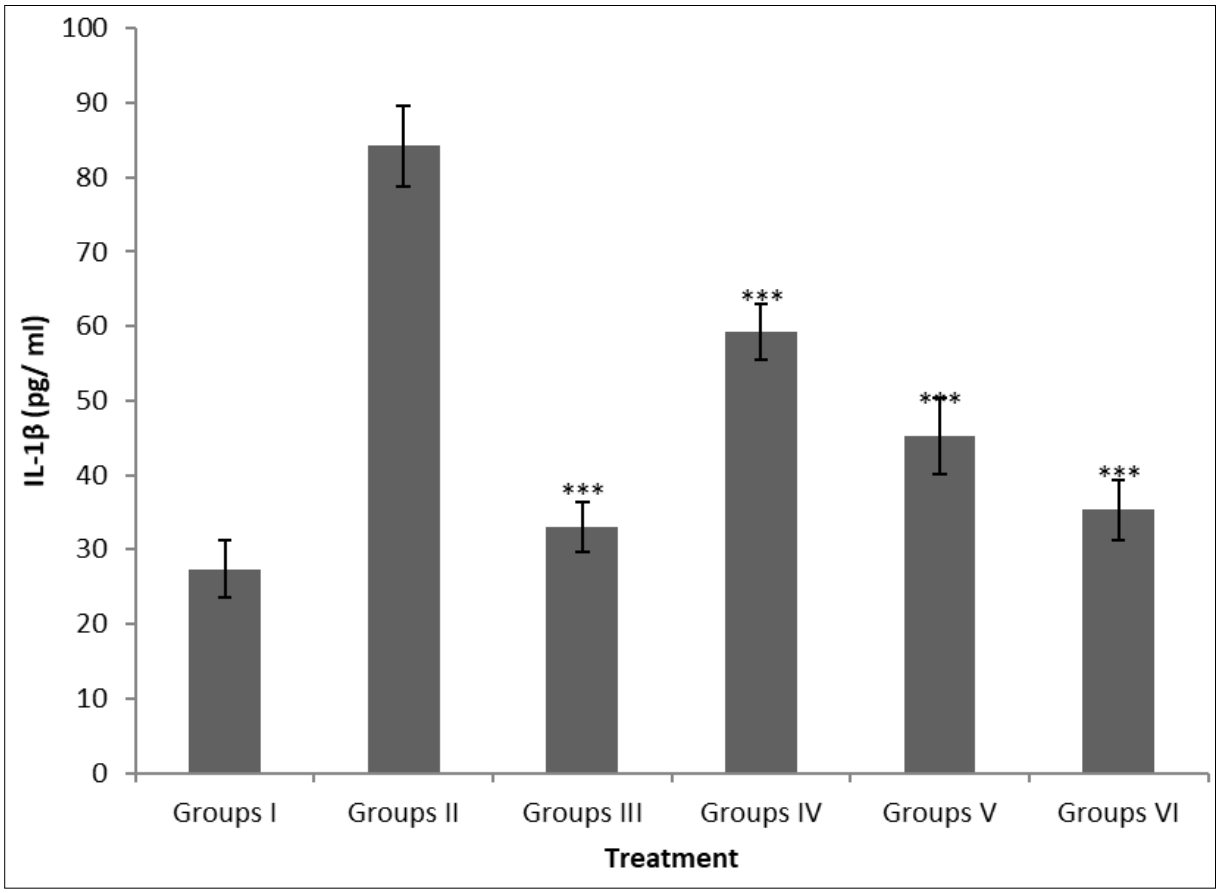

Fig 8: Effect of ascorbic acid administration on IL-1β levels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group.

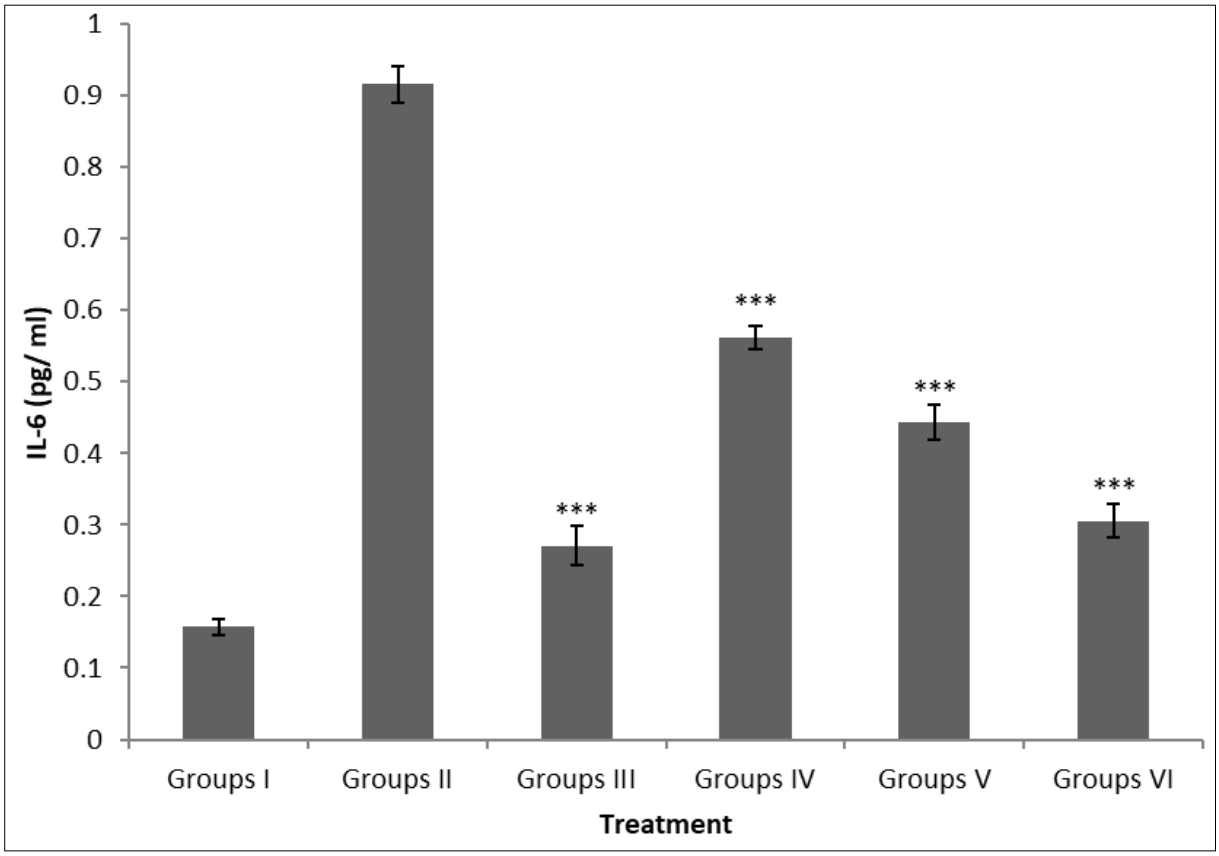

Fig 9: Effect of ascorbic acid administration on IL-6levels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group.

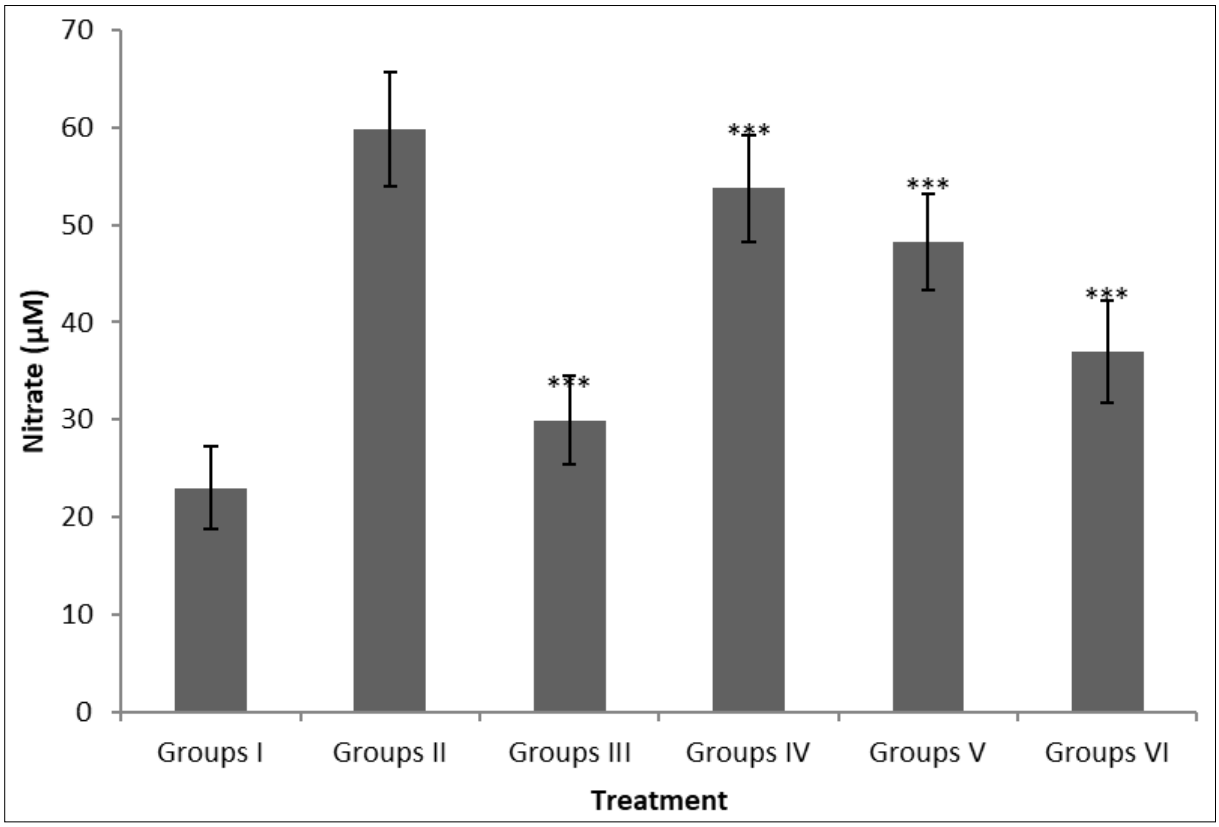

Fig 10: Effect of ascorbic acid administration on nitratelevels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group.

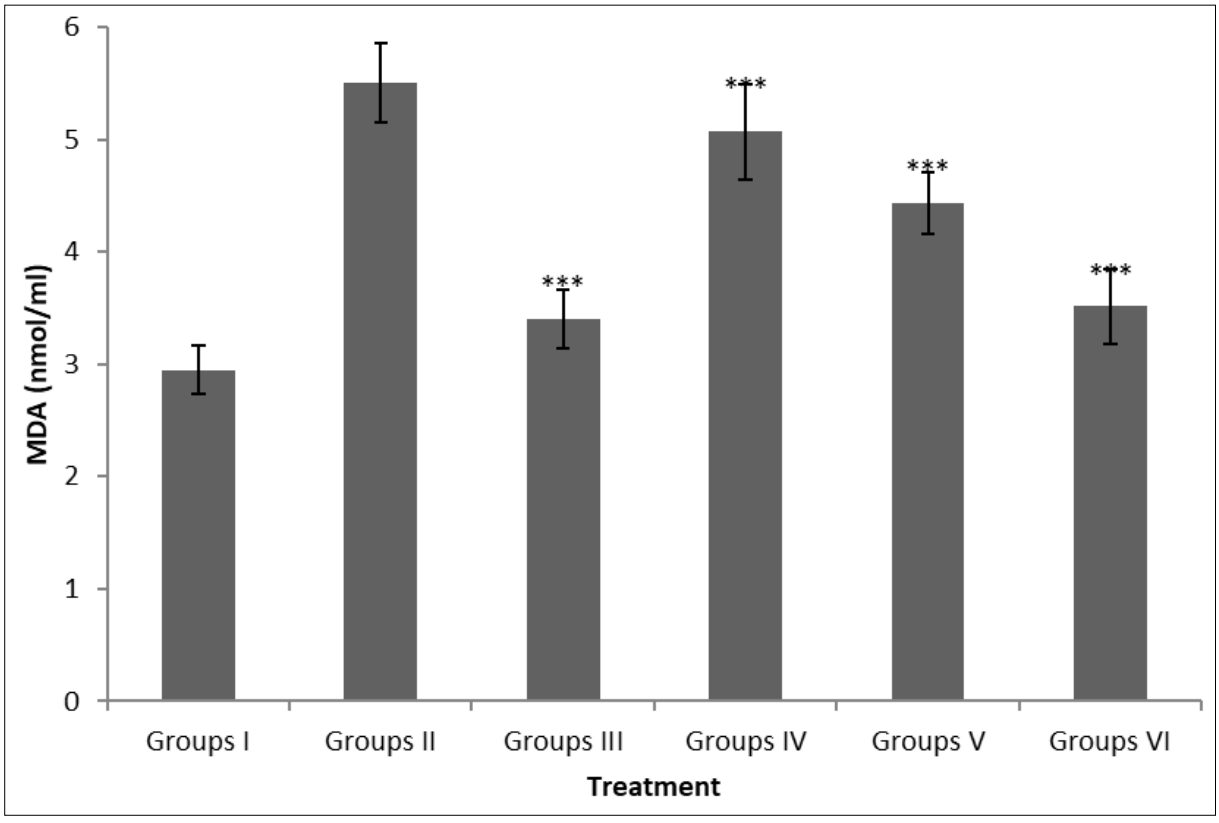

Fig 11: Effect of ascorbic acid administration on MDAlevels in isoniazid-rifampicin treated rats. Results are given as mean ± SEM of six animals in each group.

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