Multi-organs toxicities induced by anti-tubercular drugs: A preclinical experimental study

Akheruz Zaman Ahmed
Manipal Academy of Higher Education
Nandini Prashanth Bhat
Manipal Academy of Higher Education
Sneha Guruprasad Kalthur (sneha.guruprasad@manipal.edu)
Manipal Academy of Higher Education

Research Article

Keywords: Anti-tubercular drugs, Drug susceptible drugs, INH resistance drugs, RIF resistance drugs, MDR (Multidrug resistance) drugs

Posted Date: May 25th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1675716/v1

Abstract

This study was aimed to observe the multiple organ toxicities induced by different anti-tubercular drug resistant groups like isoniazid resistance, rifampicin resistance, multidrug resistance including drug susceptible anti-tubercular drugs and their time of recovery from the toxicities induced by these drugs. Wistar Albino mice were treated with drug susceptible anti-tubercular drug (Isoniazid, Rifampicin, Ethambutol & Pyrazinamide), Isoniazid resistance anti-tubercular drugs (Kanamycin, Levofloxacin, Rifampicin, Pyrazinamide & Ethambutol), Rifampicin resistance anti-tubercular drugs (Kanamycin, Levofloxacin, Ethionamide, Cycloserine, Isoniazid, Pyrazinamide & Ethambutol), Multidrug resistance anti-tubercular drug (Kanamycin, Levofloxacin, Ethionamide, Cycloserine, Pyrazinamide & Ethambutol). Animals were sacrificed after giving two weeks recovery gap from the last drug treatment. To understand the late effects, mice were sacrificed after giving two months recovery gap from the last drug treatment. Histopathology of internal organs was done by H & E and PAS. Bone marrow cells were stained with Acridine Orange dye to detect micronucleus and apoptotic cells. Expression of γ-H2AX in bone marrow cells were done by immunohistochemistry. In liver moderate to severe type of toxicities were observed in all groups in the form of nuclear alteration, sinusoidal dilation, vacuolization, necrosis, WBC infiltration. In kidney we had observed mesangial cell proliferation, vacuolization, WBC infiltration, dilation of Bowman's space. The severity of toxicity was more at short term recovery group than long term recovery groups. Drug susceptible anti-tubercular drugs (INH, RIF, and EMB & PZA) had shown more toxic in compared to other anti-tubercular drugs.

Introduction

Tuberculosis is an infection that affects around one-third of the world's population and leads to millions of deaths around the globe [1]. It also accounts for the highest mortality and morbidity worldwide. As per the 2019 statistics, a total of 1.4 million people died from this disease (WHO). However, due to the effective management and treatment involving DOTS (Directly Observed Treatment, Short Course) strategy, the global TB incidence is falling at a rate of 2% per year. World Health Organization (WHO) recommends that TB patients should have six months of TB drug treatment. This should consist of a two month "intensive" treatment phase followed by a four month "continuation" phase. First line anti-tubercular drugs (ATDs) used in DOTS strategy for human are Isoniazid (INH), Rifampin (RIF), Ethambutol (EMB), Pyrazinamide (PZA). Several studies in the past have demonstrated that ATD are cytotoxic in nature and can affect the normal tissue function [1–6]. For instances, hepatotoxicity is very common consequences as a result of ATDs treatment. There are several studies in the literatures suggesting that ATDs have the potential to cause liver injury leading to hepatitis [2, 5, 7–8]. Other than hepatotoxicity, ATDs also results in nephrotoxicity [9], neurotoxicity [10], phototoxicity [11], ocular toxicity [12–13] reproductive toxicity [14–15].

DOTS are an interventional strategy recommended by World Health Organization which uses different combination of drugs to treat TB. It is the most efficient and cost-effective method due to which the mortality rate has decreased. Various types of drugs used in DOTS strategy are majorly classified based

on their action against Mycobacterium Tuberculosis. Earlier studies have shown that the cytotoxicity of the first line ATDs can be more severe when they are used in combination [9].

The present investigation is an effort to understand the extent of organ toxicity induced by the ATDs used in Swiss albino mice as an experimental model.

Materials And Methods

Animals

60 adult female Swiss Albino mice (age: 6-8 weeks, body weight: 25-30 g) bred in Central Animal House, Manipal, Manipal Academy of Higher Education (MAHE) were housed in separate polypropylene cages. Animals were kept at temperatures (22-24° C), 12-hour light/12-hour dark cycle and 40%-60% relative air humidity under standard conditions. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC /KMC/01/2019), and experiments were conducted in accordance with the ethical standards approved by the Ministry of Social Justice and Empowerment (Government of India) and the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Experimental deign

Six to eight weeks old Swiss Albino female mice (n=60) were used as experimental model in this study. The animals were divided into short- and long-term groups. In short term animal groups, after finishing drug treatment two weeks recovery gaps were given and in long term animal groups, two months recovery gaps were given after the completion of treatment. During the recovery gap period the animals were kept under observations for any others major anomalies and were provided with regularly normal standard food pellet diet with water.

Study deigns for short term animal groups

In this experiment, 30 adult Swiss albino female mice (age: 6-8 weeks, body weight: 25-30 g) were divided into five groups (n=6/group) and treated as follows.

Normal control: All animals were treated with 0.9 % NaCl, 1 mL/kg.b.w.

Drug susceptible (DS) treatment group: In intensive phase animals were treated with INH (39 mg/kg), RIF (78 mg/kg), EMB (156 mg/kg) and PZA (195 mg/kg) once daily for one week and followed by continuation phase with INH (39 mg/kg), RIF (78 mg/kg) and EMB (156 mg/kg) once daily for three weeks.

INH resistance (INHR) treatment group: In intensive phase animals were treated with Kanamycin (Km) (97.5 mg/kg), Levofloxacin (Lfx) (130 mg/kg), RIF (78 mg/kg), PZA (195 mg/kg), EMB (156 mg/kg) once

daily for one week and followed by continuation phase Lfx (130 mg/kg), RIF (78 mg/kg), PZA (195 mg/kg) and EMB (156 mg/kg) once daily for three weeks.

RIF resistance (RIFR) treatment group: In intensive phase animals were treated with Km (97.5 mg/kg), Lfx (130 mg/kg), Ethionamide (Eto) (97.5 mg/kg), Cycloserine (Cs) (97.5 mg/kg) , INH (39 mg/kg) , PZA (195 mg/kg), EMB (156 mg/kg) once daily for one week and followed by continuation phase Lfx (130 mg/kg), Eto (97.5 mg/kg), Cs (97.5 mg/kg) , EMB (156 mg/kg) and INH (39 mg/kg) once daily for three weeks.

Multidrug resistance (MDR) treatment group: In intensive phase animals were treated with Km (97.5 mg/kg), Lfx (130 mg/kg), Eto (97.5 mg/kg), Cs (97.5 mg/kg), PZA (195 mg/kg), EMB (156 mg/kg) once daily up to one week and followed by continuation phase Lfx (130 mg/kg), Eto (97.5 mg/kg), Cs (97.5 mg/kg), EMB (156 mg/kg) once daily for three weeks.

Animals were sacrificed after giving two weeks recovery gap from the last drug treatment. To understand the late effects, mice were sacrificed after giving two months recovery gap from the last drug treatment.

Sample collection

At the end of experiment for both short and long term, animals were sacrificed and dissected to observe internal organs like liver, kidney and spleen were collected for histopathology study. Bone marrow cells were collected from the animals to assess the DNA damage.

Histopathological evaluation of liver, kidney and spleen tissue

Liver, kidney and spleen tissue were washed in normal saline 0.9% NaCl and were fixed in 10 % formalin. Then the tissues were dehydrated by putting in different graded of alcohol and then the tissues were impregnated with molten paraffin wax and sections were taken at 5 µm for staining with Hematoxylin and Eosin (H&E) and Periodic acid-Schiff stain (PAS). Extent of damage to the organ was assessed using modified histological assessment index (HAI) given by [9]. The level of lesion depends on its pathological importance. Every alteration is assessed using a score value ranging from (0 to 6) depending upon the degree and extent of the alteration. Three views per section were observed from six animals of a group. For calculating organ alteration index, formula given by [9] was used. This index represents degree of damage to an organ. It is sum of multiplied importance factors and score values of all changes found within the examined organ. A high index indicates high degree of damage.

Histopathology of liver tissue

After preparation of block on molten paraffin wax, 5 µm sections was taken at microtone and was stained with H & E to observe the pathological changes for both short and long-term groups. Prime focus was given on necrosis, nuclear alteration, WBC infiltration, vacuolization and sinusoidal dilation. All the observations were done in blinded manner. Scoring of Kupffer cells were also done under compound microscope.

Morphometric measurement of liver tissue

By using image pro software, measurements were taken on central vein diameter and sinusoidal diameter. All the data was presented in graph.

Histopathology of kidney tissue

After preparation of block on molten paraffin wax, 5 µm sections was taken and staining was done with H & E to observe the pathological changes for both short and long-term groups. The main importance was given on mesangial cells proliferation, vacuolization, WBC infiltration and dilation of Bowman's space. All the observation was done in blinded manner.

Morphometric measurement of kidney tissue

By using image pro software, measurements were taken for glomerular diameter, Bowman's space diameter and Bowman's capsule diameter. All the data were presented in graph.

Histopathology of spleen tissue

After preparation of block on molten paraffin wax, 5 µm sections was taken at microtone and was stained with H&E to observe the pathological changes for both short and long-term groups of spleen tissue.

Collection of bone marrow cells

Both the femur bone was collected from all short-term animals. By using 1 mL syringe, PBS (Phosphate Buffered Saline) was injected from one end of femur bone and from other end bone marrow cells were collected in 2 mL eppendorf tube. The tube was centrifuged three times at 2000 rpm for 10 minutes to get clean sediment. Each time supernatant was discarded and the sediments were mixed with PBS and finally bone marrow slides were made by spreading the sediments on glass slide.

Expression of γ-H2AX in bone marrow cells to assess the DNA damage

The bone marrow cells were washed and fixed in 4 % paraformaldehyde overnight. The supernatant was discarded after centrifuging the cells at 1000 rpm for 10 min. The pellet was washed in PBS three times to remove the traces of fixative. The cells were incubated with permeabilization buffer containing 0.25 % Triton X-100 in PBS for 15 min at room temperature. After centrifugation, the cell pellet was incubated with blocking solution containing 1 % bovine serum albumin in PBS with 0.1 % Tween-20 for 30 min at 37 ºC. The cells were incubated with 1:50 anti-phospho histone H2AX antibody (primary antibody) in blocking solution at 4 ºC. The cells were washed again with PBS and incubated with 1:200 goat anti-rabbit IgG H&L Alexa Fluor 488 (secondary antibody) for 2 h at room temperature. The cells were washed three times with PBS, transferred to clean grease free microscopic slides and were mounted using Fluoroshield™ with DAPI. A clean coverslip was placed on the cells and observed under fluorescence microscope (Zeiss Imager A1-AX10) at 400x magnification for the signals. Total 500 cells were counted from each group to assess the DNA damage.

Micronucleus assay

After preparation of bone marrow slides, the slides were stained with Acridine Orange dye. The scoring was done according to the criteria given by [16]. The nucleus was considered as a micronucleatic cells that were present inside the cytoplasm and counted as micronucleus if the size $1/3^{rd}$ of the main nucleus. Scoring was done for 2000 lymphocytes.

Apoptotic cells assay

After preparation of bone marrow slides the section was stained with Acridine Orange dye to observe apoptotic cell. Identification of apoptotic cell was done according to the following criteria; cell shrinkage, chromatin condensation, DNA fragmentation, membrane blabbing and formation of apoptotic bodies. All the scoring was done in blinded manner and the data was presented in graph.

Statistical analysis

Using the Statistical Package for the Social Sciences (SPSS version 16.0; SPSS), data was expressed as mean ± standard deviation and analyzed by one way analysis of variance (ANOVA) followed by post hoc Tukey test. A level for $p \leq 0.05$ was considered to be statistically significant ($p \leq 0.05$).

Results

In the present study, cytotoxicity induced by ATDs in liver, kidney, spleen & bone marrow of adult Swiss albino mice were evaluated. Further, short term and long-term tissues were compared for cytotoxicity and severe histological alteration was found on short term animals group where only two weeks recovery gap was given and in long term treated animals the degree of cytotoxicity gradually decreased where two month recovery gaps were given.

Effects of ATDs on liver of short-term group animals

In short term recovery gap, DS group had shown severe necrosis and severe sinusoidal dilation with moderate nuclear alteration, WBC infiltration and vacuolization. The INHR group showed moderate nuclear alteration, WBC infiltration, vacuolization and sinusoidal dilatation. The RIFR group exhibited moderate sinusoidal dilation with mild type nuclear alteration, WBC infiltration and vacuolization whereas; MDR group had shown moderate sinusoidal dilation with mild nuclear alteration. All the comparisons were done with normal control group (Fig. 1a). PAS staining showed mild intensity in staining indicating decrease glycogen granules with basement membrane appearing normal (Fig. 1c).

Effects of ATDs on liver of long-term recovery groups

In long term recovery gap, DS group showed mild necrosis, sinusoidal dilation, nuclear alteration, WBC infiltration and vacuolization in comparison with normal control group. The INHR, RIFR and MDR group

had mild necrosis, nuclear alteration, WBC infiltration, vacuolization and sinusoidal dilatation in comparison with normal control group (Fig. 1b).

Morphometric measurement of liver tissue:

Effect of ATDs on diameter of central hepatic veins:

There was significant increase in diameter of central hepatic vein in DS ($p<0.001$), INHR ($p<0.001$), RIFR ($p<0.001$) and in MDR ($p<0.001$) group in comparison with normal control group. The diameter of hepatic central vein significantly decreased in INHR ($p=0.02$), RIFR ($p=0.03$) and in MDR ($p=0.04$) in comparison with DS treatment group (Fig. 4b).

Effect of ATDs on sinusoidal dilation:

Significant increase in sinusoidal dilation was observed in DS ($p<0.001$), INHR ($p=0.04$), RIFR ($p=0.03$) and MDR ($p<0.001$) group in compared with normal control group. It significantly decreased in INHR ($p<0.001$) and in RIFR ($p<0.001$) in compared with DS treatment group. It significantly increased in MDR ($p=0.003$) in compared with INHR group and in MRD ($p=0.003$) in comparison with RIFR group (Fig. 4c).

Effect of ATDs on Kupffer cell number:

The number of Kupffer cells significantly increased in DS ($p=0.003$), INHR ($p=0.04$), and RIFR ($p=0.002$) group in compared with normal control group. The number was significantly decreased in MDR ($p=0.04$) in compared with DS treatment group and in MDR ($p=0.004$) in compared with RIFR group (Fig. 4d).

Effect of ATDs on kidney tissue in short term recovery gap

The DS group showed severe WBC infiltration and dilation of Bowman's space with moderate type mesangial cell proliferation and vacuolization. INHR group showed severe WBC infiltration with moderate type mesangial cell proliferation, vacuolization and dilatation of Bowman's space. RIFR group showed severe WBC infiltration with moderate type mesangial cell proliferation, vacuolization and dilatation of Bowman's space. MDR group had severe WBC infiltration with moderate type mesangial cell proliferation, vacuolization and dilatation of Bowman's space (Fig. 5a). All the comparisons were done with normal control group.

Effect of ATDs on kidney tissue in long term recovery gap:

The DS group showed mild WBC infiltration and dilation of Bowman's space with moderate type mesangial cell proliferation and vacuolization. INHR group showed mild WBC infiltration, mesangial cell proliferation, vacuolization and dilatation of Bowman's space. The RIFR group showed mild WBC infiltration, mesangial cell proliferation, vacuolization and dilatation of Bowman's space. Mild WBC infiltration, mesangial cell proliferation, vacuolization and dilatation of Bowman's space were observed in MDR group (Fig. 5b).

Effect of ATDs on glomerular diameter:

Glomerular diameter significantly decreased in DS ($p<0.001$), INHR ($p=0.008$), RIFR ($p<0.001$) and in MDR ($p=0.009$) in comparison with normal control group. It significantly increased in MDR ($p=0.004$) group in compared with RIFR and INHR ($p=0.04$) and MDR ($p=0.003$) in compared with DS treatment group (Fig. 8 b).

Effect of ATDs on Bowman's capsule diameter:

There was significance increase of Bowman's capsule diameter in DS ($p<0.001$), INHR ($p=0.006$) and in RIFR group ($p<0.001$) in comparison with normal control group. Bowman's capsule diameter significantly decreased in INHR ($p=0.007$), RIFR ($p=0.03$) and in MDR ($p<0.001$) in compared with DS treatment group (Fig. 8 c).

Effect of ATDs on Bowman's space diameter:

Bowman's space diameter significantly increased in DS ($p<0.001$), INHR ($p<0.001$), RIFR ($p<0.001$) and MDR ($p=0.03$) in compared with normal control group and it significantly decreased in MDR ($p=0.02$) in compared with DS treatment group (Fig. 8 d).

Effect of ATDs on spleen:

White pulp-hyper cellularity was seen in all drug treated groups due to depletion in the B lymphocytes and hyalinization of vessels due to adverse effect of ATDs. Red pulp - extra medullary hematopoiesis and small hemorrhagic areas was seen in treatment group (Fig. 9).

Effect of ATDs on white pulp diameter of spleen

There was significant increase in white pulp diameter of spleen in DS ($p<0.001$) and INHR ($p=0.004$) group in compared with normal control group. White pulp diameter significantly decreased in INHR ($p=0.008$), RIFR ($p=0.009$) and in MDR ($p=0.005$) in compared with DS treatment group (Fig. 10b).

Central arteriole diameter significantly increased in DS ($p<0.001$), INHR ($p=0.003$) and in RIFR ($p=0.003$) group in compared with normal control group. It significantly decreased in INHR ($p<0.001$), RIFR ($p<0.001$) and in MDR ($p<0.001$) in compared with DS group (Fig. 10 c)

Effect of ATDs on the DNA damage in bone marrow by γH2AX

After treating the animals with ATDs, the bone marrow from control group had 5.36 ±1.35 % of γH2AX positive cells which was observed to be increased in all the treatment groups with highest positive cells in INHR treatment group (10.86 ± 3.69 %). The other groups DS, RIFR and MDR had 8.50 ± 1.33 %, 7.04 ± 0.82 % and 7.12 ± 1.51 % of positive cells in bone marrow cells. Even though there was an increase in the percentage of positive cells it was not statistically significant (Fig. 10d).

Effect of ATDs on the DNA integrity of bone marrow cells:

To detect bone marrow cytotoxicity induced by ATDs we had done apoptotic and micronucleus assay.

There was significant increase of apoptotic cell numbers in DS group ($p<0.001$), INHR ($p<0.001$) and in RIFR ($p<0.001$) group in compared with normal control group. Apoptotic cells count significantly decreased in INHR ($p<0.001$), MDR ($p<0.001$) in compared with DS group and in MDR ($p<0.001$) compared with RIFR group (Fig. 11).

Micronucleatic cell counts significantly increased in DS ($p<0.001$), INHR ($p=0.003$), RIFR ($p<0.001$) and in MDR ($p=0.005$) in compared with normal control group. It significantly decreased in INHR ($p<0.001$), MDR ($p<0.001$) in compared with DS treatment group (Fig. 12).

Discussion

Oxidative stress induced by anti-tubercular drugs treatment leads to the production of free radicals mainly reactive oxygen species (ROS) that causes mitochondrial dysfunction and activation of apoptotic pathway by releasing cytochrome C from mitochondria [17–19]. Oxidative stress, mitochondrial dysfunction and activation of apoptotic pathway by ATDs are the key mechanisms in histopathological alteration in hepatocytes induced by anti-tubercular drugs [17–19].

Free radicals are formed from the reactive metabolites of anti-tubercular drugs cause lipid peroxidation that will lead to degradation endoplasmic reticulum & membrane lipids which are high source of poly unsaturated fatty acids (PUFA). This will lead to the formation of malonaldehyde (MDA) which have the ability to cause loss of cell membrane integrity and can damage hepatocytes [9]. In rats toxicant group (anti-tubercular drugs treated group) due to increased lipid peroxidation, results in anti-oxidant defence system to clear all free radicals induced by anti-tubercular drugs [20]. Though liver has remarkable ability for the elimination of toxins, in other side this elimination of toxins will cause damage to liver cells as well [21]. Due to high vulnerability of cell membrane of hepatocytes to anti-tubercular drugs, it will lead to per oxidative damage to the cell membrane. The PZA, in along with INH and RIF, is also associated with an increased incidence of toxicity [22].

Anti-tubercular drug causes liver toxicity and patchy necrosis in all animals treated with anti-tubercular drugs [23]. The present study also reported liver toxicity with patchy necrosis in drug treated groups. In DS groups the degeneration was more due to combined used of first line anti-tubercular drugs.

First-line anti-tuberculosis drugs causes hepatotoxicity on Wistar rats along with severe necrosis, vacuolization and WBC infiltration [9]. In corroborating with all these previous finding this present study also had shown severe necrosis, vacuolization and WBC infiltration. Vacuolization and necrosis might be due to free radicals, excessive production of oxidative stress marker mainly MDA and TNF-α that can degenerate cell wall and causes blabbing of cell walls. WBC infiltration might be due to activation of inflammatory pathway activated by anti-tubercular drugs.

Though, very few studies had reported the measurement of glomerular diameter, sinusoidal dilatation and number of Kupffer cells in anti-tubercular treated Wistar albino mice. In this study we found significant change in glomerular diameter, sinusoidal dilation and number of Kupffer cells per field in all drug treated group as compared to normal control.

Nephrotoxicity is an adverse side effect of many drugs mainly non-steroidal anti-inflammatory drugs, antihypertensive including anti-tubercular drugs. Many studies reported that anti-tubercular drugs cause relative glomerular shrinkage and dilation of Bowman's space [9]. In this present study also shrinkage of glomerular diameter, dilation of Bowman's space was seen. Similar to our results Shabana et al. reported frequent shrinkage of glomerular capillaries associated with Bowman's space dilatation and cellular proliferation in a mesangial area [24]. Previous study reported inflammatory changes in the glomeruli of albino rats after 60 days of rifampicin treatment [25]. All these previous study reports corroborated with the findings of the present study.

Earlier study found severe infiltration in the glomerulus in kidneys of rifampicin-isoniazid treated Wistar rats [26]. In our present study also necrosis, glomerular congestion, WBC infiltration were observed in all drug treated group that indicate nephrotoxicity and degree of nephrotoxicity was more in DS group. In this present study H&E and PAS-stained kidney slides showed several alterations and the most prominent findings were glomerular changes. Renal corpuscles appeared enlarged with mild mesangial hypertrophy. However, glomerular capillary tufts revealed shrinkage resulting in noticeably dilated Bowman's space. Interstitial edema was mild, visible mostly around larger blood vessels and accompanied with peritubular capillary dilatation were also seen.

Oral administration of first line of anti-tubercular drugs on experimental animal causes many alterations in spleen like lack of demarcation of red and white pulp, WBC infiltration in many places. It also causes many chromosomal alterations like chromatid breaks, centromere fusion, elongated chromosomes, wooly chromosomes was observed by [9]. In the present study spleen showed hyper-cellularity in white pulp with increased diameter of central arteriole indicating hyalinization of vessels due to toxic effect of ATT. Red pulp indicated haemopoesis suggesting splenomegaly induced by anti-tubercular drug treatment.

Masjed et al. observed chromosomal aberrations and micronuclei in lymphocytes of patients before and after exposure to ATDs. They found significantly high micronucleatic cells in exposed group in comparison to un-exposed group [27]. This present study also presented significantly high number of micronucleatic cells in drug treated group as compared to control.

To confirm cytotoxic effect of ATDs on bone marrow, apoptotic cells assay was done. Cytotoxic drugs under long term treatment results in production of micronucleus as well as apoptotic cells body in bone marrow due to excessive production of cytochrome from mitochondrial dysfunction. In this present study maximum number of apoptotic and micronucleatic cells were observed in DS group that indicate DS group more cytotoxic than other drug group. Many studies had shown that when first line anti-tubercular drugs mainly RIF, INH and PYZ are used in combined they show more cytotoxic effect [9]. In this study DS group RIF, INH and PYZ were used and had shown comparatively higher cytotoxicity than other group.

Conclusion

Drug susceptible groups are potentially hepatotoxic to Swiss albino female mice showing severe necrosis, vacuolization as compared to other groups. Drug susceptible groups are more cytotoxic to bone marrow cells produces more micronucleatic and apoptotic cells. From this study we conclude that drug susceptible anti-tubercular drugs (INH, RIF, EMB & PZA) are more toxic when these are used in combined for long duration.

Declarations

Acknowledgement

This work was financially supported by Manipal Academy of Higher Education, Manipal (India) for which the authors are grateful.

Conflict of Interest

The authors have no conflicts of interest that are directly relevant to the content of this manuscript.

References

1. Martin SJ, Sabina EP (2016) Amelioration of anti-tuberculosis drug induced oxidative stress in kidneys by Spirulina fusiformis in a rat model. Ren Fail 38:1115–1121. https://doi.org/10.1080/0886022X.2016.1184940
2. Chang KC, Leung CC, Yew WW, et al (2008) Hepatotoxicity of pyrazinamide: Cohort and case-control analyses. Am J Respir Crit Care Med 177:1391–1396. https://doi.org/10.1164/rccm.200802-355OC
3. Bondarenko LB, Shayakhmetova GM, Byshovets TF, Kovalenko VM (2011) Pyrazinamide-mediated changes in rat type I collagen and spermatogenesis indices. Acta Pol Pharm - Drug Res 68:285–290
4. Rao A, Nayak G, Kumari S, et al (2016) Ethambutol induces testicular damage and decreases the sperm functional competence in Swiss albino mice. Environ Toxicol Pharmacol 47:28–37. https://doi.org/10.1016/j.etap.2016.08.012
5. Tostmann A, Boeree MJ, Aarnoutse RE, et al (2008) Antituberculosis drug-induced hepatotoxicity: Concise up-to-date review. J Gastroenterol Hepatol 23:192–202. https://doi.org/10.1111/j.1440-1746.2007.05207.x
6. Ruan LY, Fan JT, Hong W, et al (2018) Isoniazid-induced hepatotoxicity and neurotoxicity in rats investigated by 1H NMR based metabolomics approach. Toxicol Lett 295:256–269. https://doi.org/10.1016/j.toxlet.2018.05.032
7. Yuan L, Kaplowitz N (2013) Mechanisms of drug-induced liver injury. Clin Liver Dis 17:507–518. https://doi.org/10.1016/j.cld.2013.07.002
8. El Bouazzi O, Hammi S, Bourkadi JE, et al (2016) First line anti-tuberculosis induced hepatotoxicity: Incidence and risk factors. Pan Afr Med J 25:1–10.

https://doi.org/10.11604/pamj.2016.25.167.10060

9. Sharma R, Kaur R, Mukesh M, Sharma VL (2018) Assessment of hepatotoxicity of first-line anti-tuberculosis drugs on Wistar rats. Naunyn Schmiedebergs Arch Pharmacol 391:83–93. https://doi.org/10.1007/s00210-017-1434-8
10. Kass JS, Shandera WX (2010) Nervous system effects of antituberculosis therapy. CNS Drugs 24:655–667. https://doi.org/10.2165/11534340-000000000-00000
11. Vargas F, Rivas C, Díaz Y, Fuentes A (2003) Photodegradation pathways and the in vitro phototoxicity of pyrazinamide, a phototoxic antitubercular drug. J Photochem Photobiol B Biol 72:87–94. https://doi.org/10.1016/j.jphotobiol.2003.09.010
12. Chatterjee VKK, Buchanan DR, Friedmann AI, Green M (1986) Ocular toxicity following ethambutol in standard dosage. Br J Dis Chest 80:288–291. https://doi.org/10.1016/0007-0971(86)90066-5
13. Kahana LM (1987) Toxic ocular effects of ethambutol. Can Med Assoc J 137:213–216
14. Shayakhmetova GM, Bondarenko LB, Voronina AK, et al (2015) Induction of CYP2E1 in testes of isoniazid-treated rats as possible cause of testicular disorders. Toxicol Lett 234:59–66. https://doi.org/10.1016/j.toxlet.2015.02.008
15. Rao A, Nayak G, Kumari S, et al (2020) Exposure to first line anti-tuberculosis drugs in prepubertal age reduces the quality and functional competence of spermatozoa and oocytes in Swiss albino mice. Environ Toxicol Pharmacol 73:103292. https://doi.org/10.1016/j.etap.2019.103292
16. Countryman PI, Heddle JA (1976) The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes. Mutat Res - Fundam Mol Mech Mutagen 41:321–331. https://doi.org/10.1016/0027-5107(76)90105-6
17. Lian Y, Zhao J, Xu P, et al (2013) Protective Effects of Metallothionein on Isoniazid and Rifampicin-Induced Hepatotoxicity in Mice. PLoS One 8:. https://doi.org/10.1371/journal.pone.0072058
18. Nicoletti NF, Rodrigues-Junior V, Santos AA, et al (2014) Protective effects of resveratrol on hepatotoxicity induced by isoniazid and rifampicin via SIRT1 modulation. J Nat Prod 77:2190–2195. https://doi.org/10.1021/np5003143
19. Chen L, Xu B, Liu L, et al (2010) Hydrogen peroxide inhibits mTOR signaling by activation of AMPKα leading to apoptosis of neuronal cells. Lab Investig 90:762–773. https://doi.org/10.1038/labinvest.2010.36
20. Naik SR, Panda VS (2008) Hepatoprotective effect of Ginkgoselect Phytosome® in rifampicin induced liver injurym in rats: Evidence of antioxidant activity. Fitoterapia 79:439–445. https://doi.org/10.1016/j.fitote.2008.02.013
21. Amir M, Khan MA, Ahmad S, et al (2016) Ameliorating effects of Tamarindus indica fruit extract on anti-tubercular drugs induced liver toxicity in rats. Nat Prod Res 30:715–719. https://doi.org/10.1080/14786419.2015.1039001
22. Mitra SK, Venkataranganna M V., Sundaram R, Gopumadhavan S (1998) Protective effect of HD-03, a herbal formulation, against various hepatotoxic agents in rats. J Ethnopharmacol 63:181–186. https://doi.org/10.1016/S0378-8741(98)00088-9

23. Eminzade S, Uraz F, Izzettin F V. (2008) Silymarin protects liver against toxic effects of anti-tuberculosis drugs in experimental animals. Nutr Metab 5:1–8. https://doi.org/10.1186/1743-7075-5-18
24. Shabana MB, Ibrahim HM, Khadre SEM, Elemam MG (2012) Influence of rifampicin and tetracycline administration on some biochemical and histological parameters in albino rats. J Basic Appl Zool 65:299–308. https://doi.org/10.1016/j.jobaz.2012.10.009
25. Issn J, Online FA (2013) Effect of rifampicin on the kidney of albino rats. J Appl Sci Environ Manag 17:125–131
26. Muzika V, Čustović S, Mornjaković Z, et al (2016) Histological study of isoniazid-rifampicin related nephrotoxicity in Wistar rats. Folia Med Fac Med Univ Saraeviensis 51:4–9
27. Masjedi MR, Heidary A, Mohammadi F, et al (2000) Chromosomal aberrations and micronuclei in lymphocytes of patients before and after exposure to anti-tuberculosis drugs. Mutagenesis 15:489–494. https://doi.org/10.1093/mutage/15.6.489

Figures

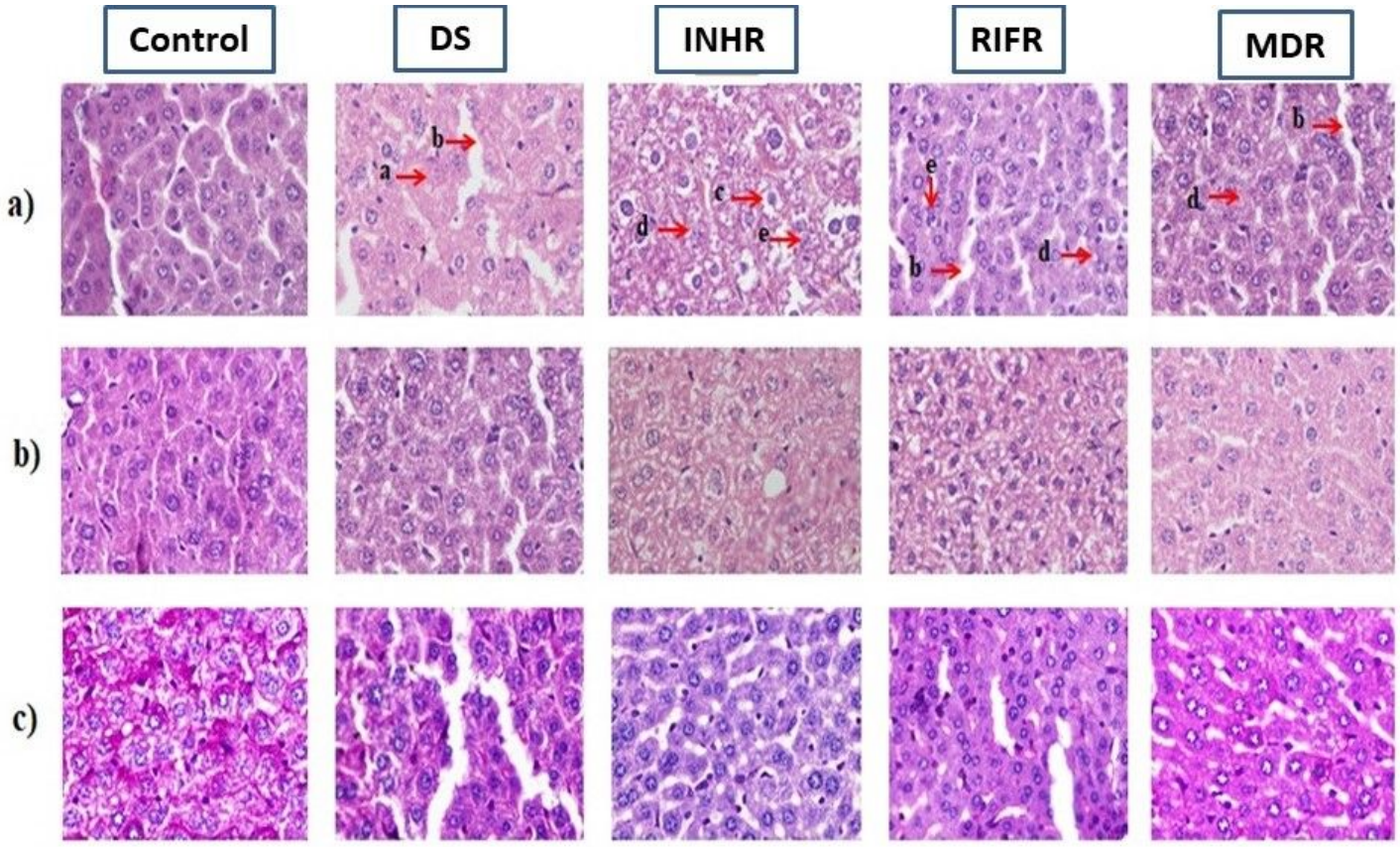

Figure 1

a) Histopathological evaluation of liver tissue from short term recovery gap groups stained with H&E (under 400X) (a- Nuclear alteration, b- Sinusoidal dilation, c- Vacuolization, d- Degenerated hepatocytes, e-

WBC infiltration). **b)** Histopathological examination of liver from long term recovery gap groups stained with H&E (under 400X) **c)** Histopathological examination of liver from short term animal groups stained with PAS (under 400X).

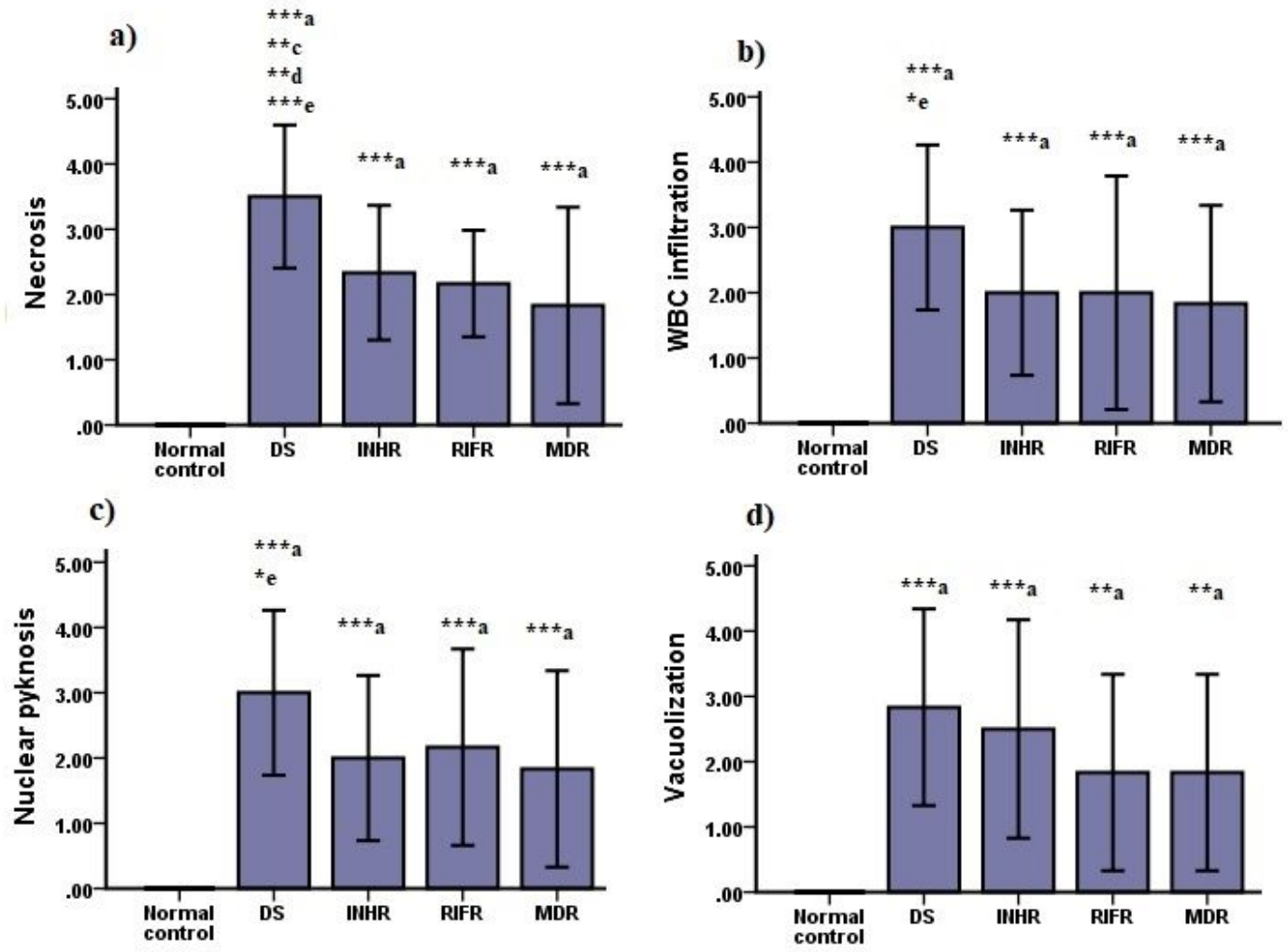

Figure 2

Scoring for short term (animals were sacrificed after two weeks from last treatment) hepatic tissues stained with hematoxylin and eosin was performed as 0 = absent; 1 = low; 2 = mild; 3 = moderate; and 4 = high or severe. Data were represented as mean ± SD, and one-way ANOVA followed by Tukey's post hoc test was done and p value less than 0.05 were taken statistically significant (***p≤0.001, **p≤0.01), ([a] compared to normal control, [c] compared to INHR, [d] compared to RIFR, [e] compared to MDR).

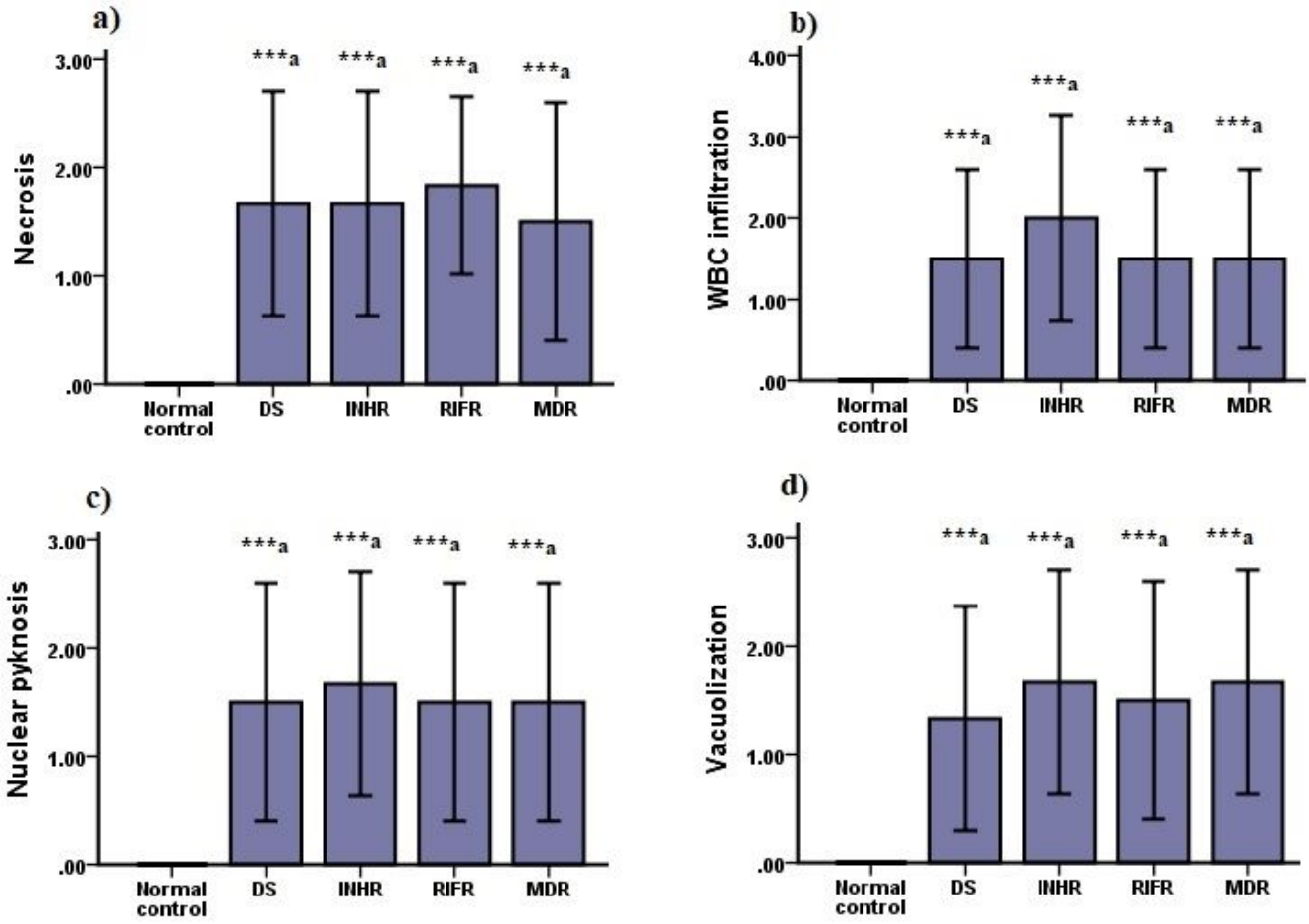

Figure 3

Scoring for long term (animals were sacrificed after two months from last treatment) hepatic tissues stained with hematoxylin and eosin was performed as 0 = absent; 1 = low; 2 = mild; 3 = moderate; and 4 = high or severe. Data were represented as mean ± SD, and one-way ANOVA followed by Tukey's post hoc test was done and p value less than 0.05 were taken statistically significant (***$p \leq 0.001$) (^{a} compared to normal control).

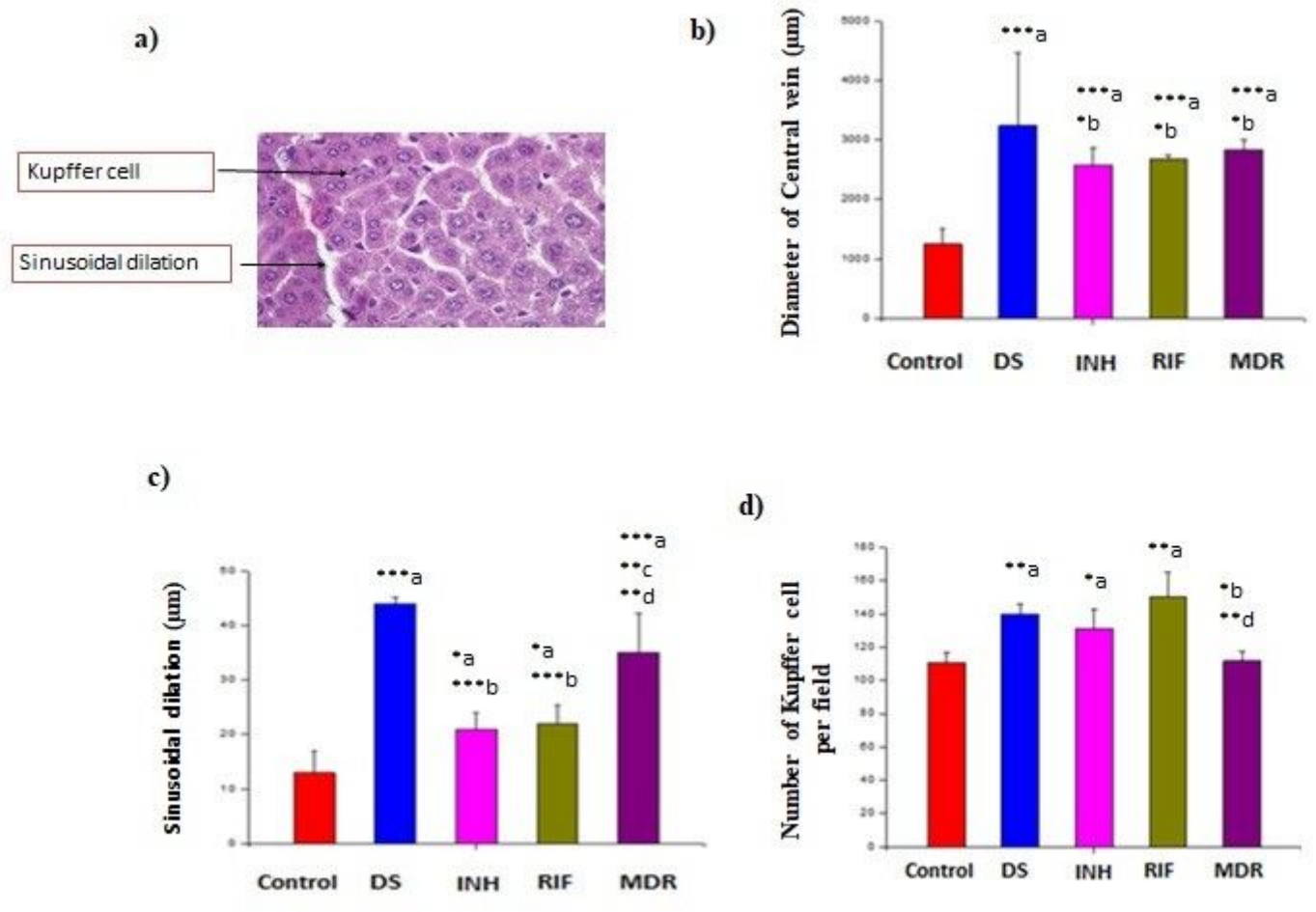

Figure 4

a) Hepatocytes showing Kupffer cells and sinusoidal dilution short term animal groups **b)** Morphometric measurement of central vein diameter short term animal groups c) Morphometric measurement of sinusoidal dilation, **d)** Quantitative analysis of Kupffer cells of short term animal groups. Data were represented as mean ± SD, and one-way ANOVA followed by Tukey's post hoc test was done and p value less than 0.05 were taken statistically significant (***p≤0.001, **p≤0.01, *p≤0.05), ([a] compared to control, [b] compared to DS, [c] compared to INHR, [d] compared to RIFR).

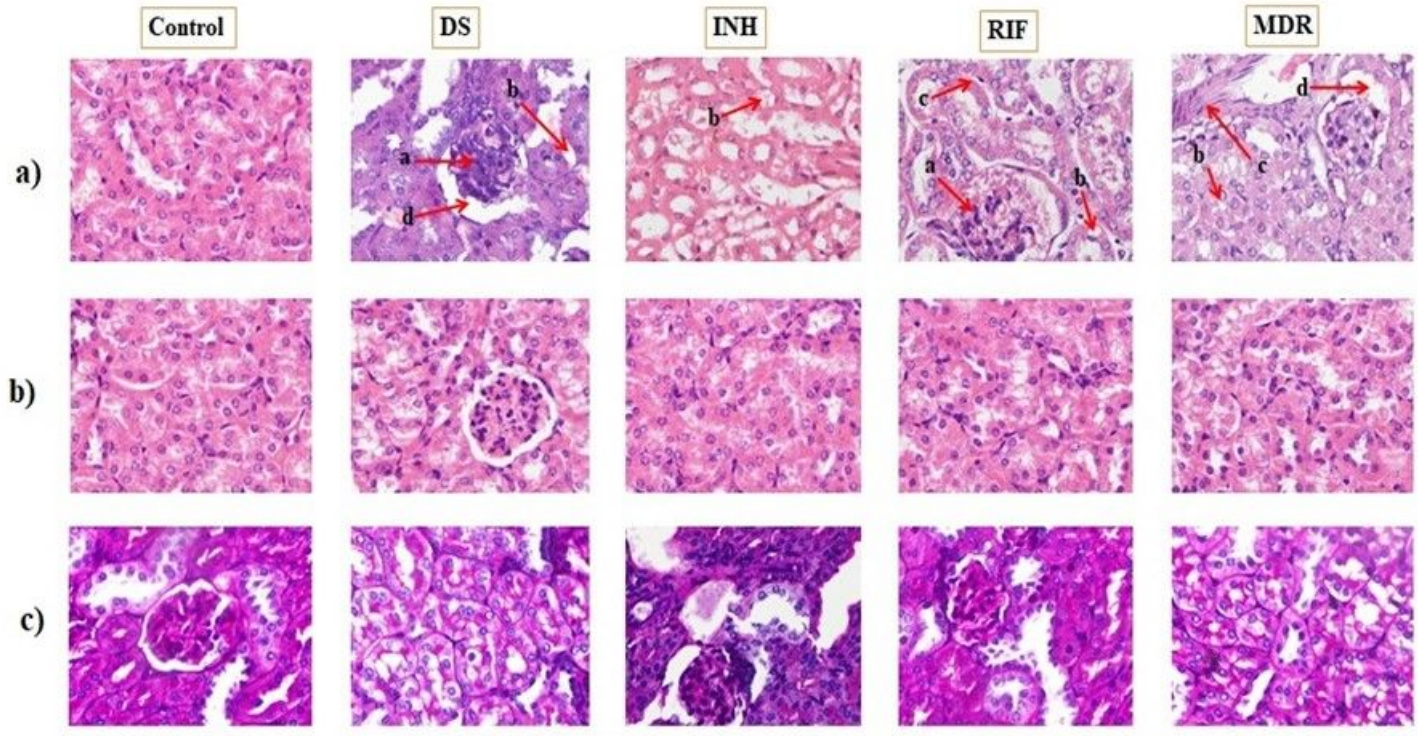

Figure 5

a) Histopathological examination of kidney tissue from short term animal groups stained with H&E (under 400X) (a- Mesangial cell proliferation, b- Vacuolization, c- WBC infiltration, d- Dilation of Bowman's space). **b)** Histopathological examination of kidney tissue from long term animal groups stained with H&E (under 400X) **c)** Histopathological examination of kidney tissue from short term animal groups stained with PAS (under 400X)

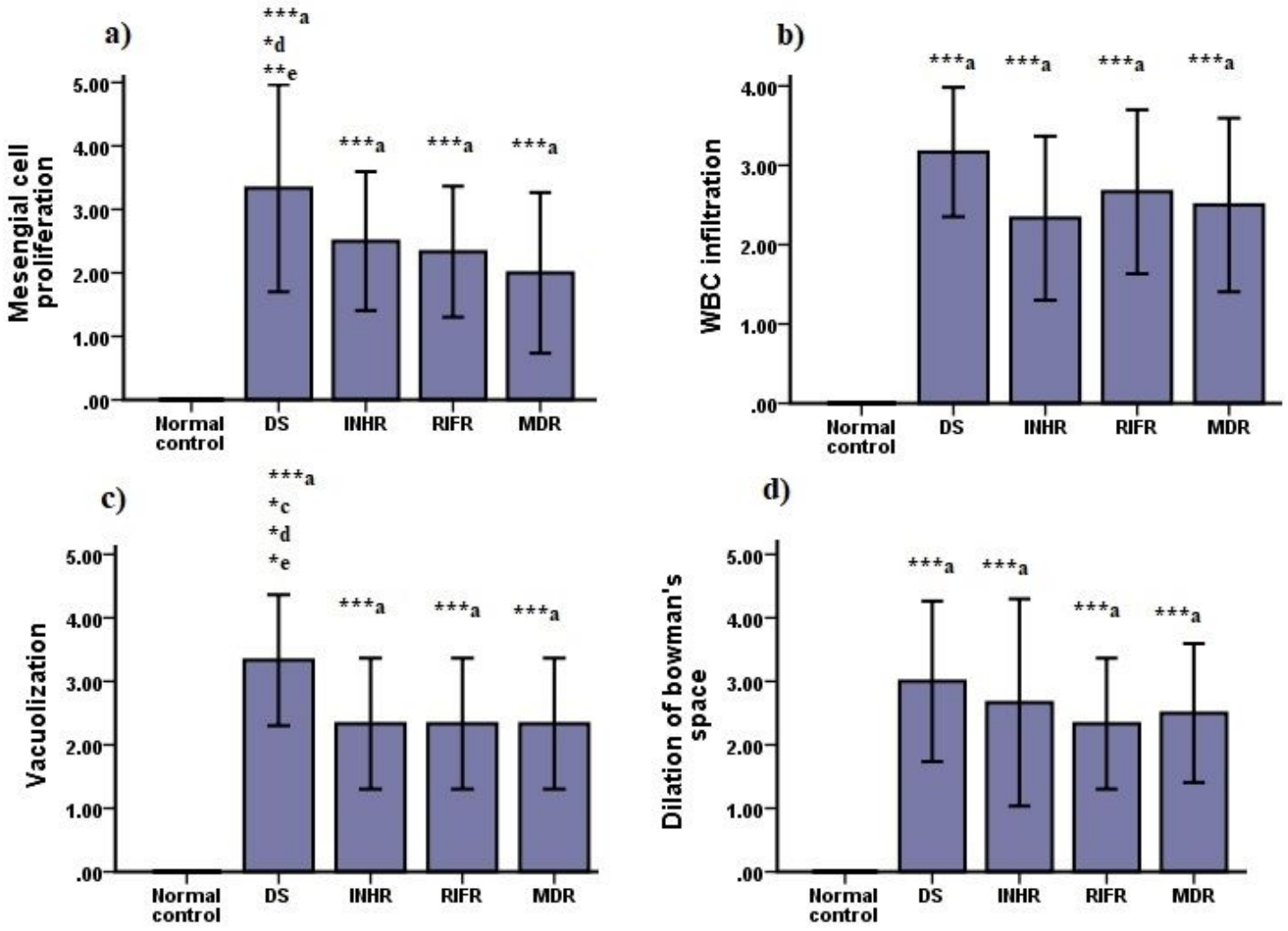

Figure 6

Scoring for short term (animals were sacrificed after two weeks from last treatment) kidney tissues stained with hematoxylin and eosin was performed as 0 = absent; 1 = low; 2 = mild; 3 = moderate; and 4 = high or severe. Data were represented as mean ± SD, and one-way ANOVA followed by Tukey's post hoc test was done and p value less than 0.05 were taken statistically significant (***p≤0.001, **p≤0.01, *p<0.05), ([a] compared to normal control, [c] compared to INHR, [d] compared to RIFR, [e] compared to MDR).

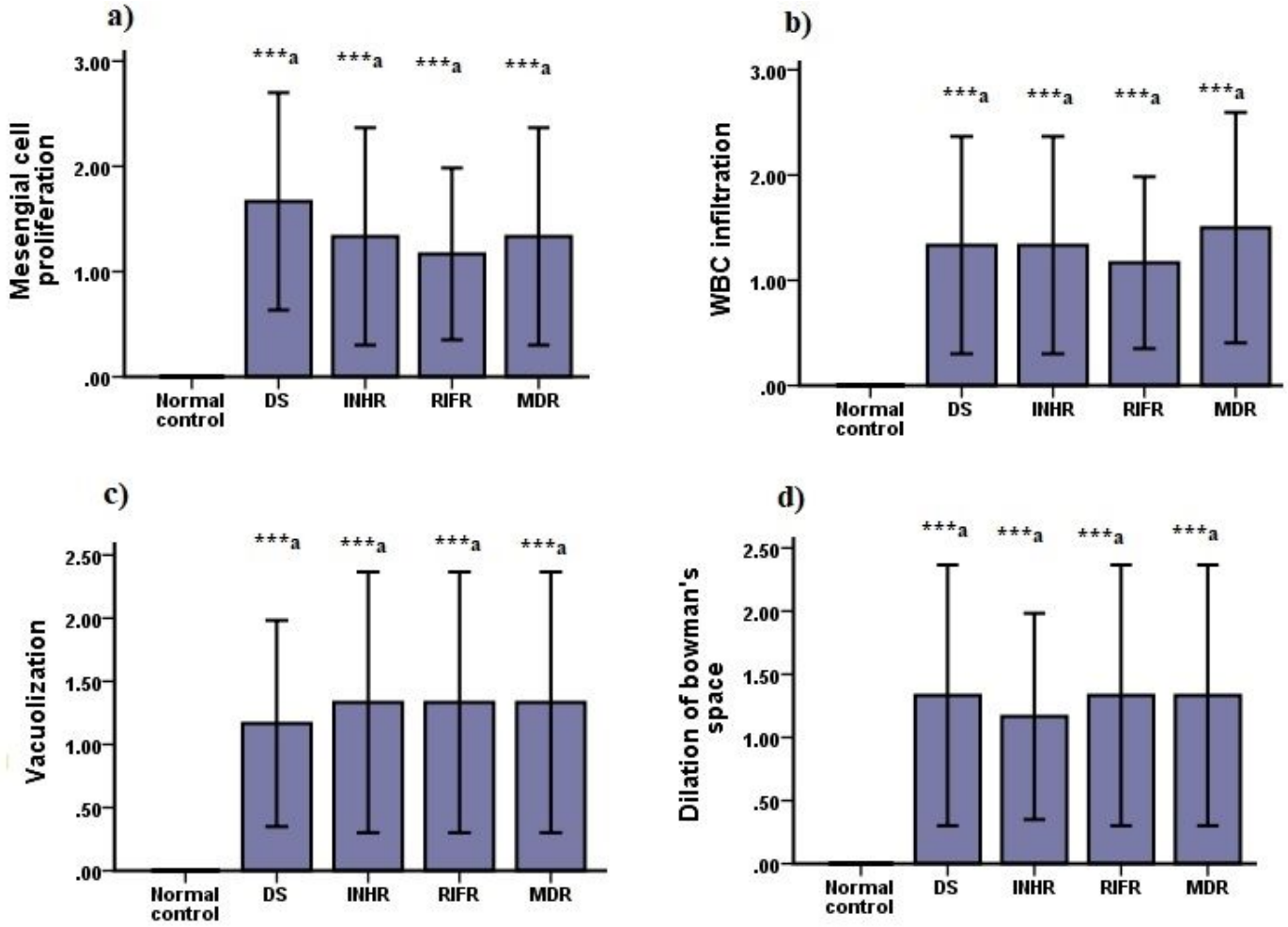

Figure 7

Scoring for long term (animals were sacrificed after two months from last treatment) kidney tissues stained with hematoxylin and eosin was performed as 0 = absent; 1 = low; 2 = mild; 3 = moderate; and 4 = high or severe. Data were represented as mean ± SD, and one-way ANOVA followed by Tukey's post hoc test was done and p value less than 0.05 were taken statistically significant (***$p \leq 0.001$), ([a] compared to normal control)

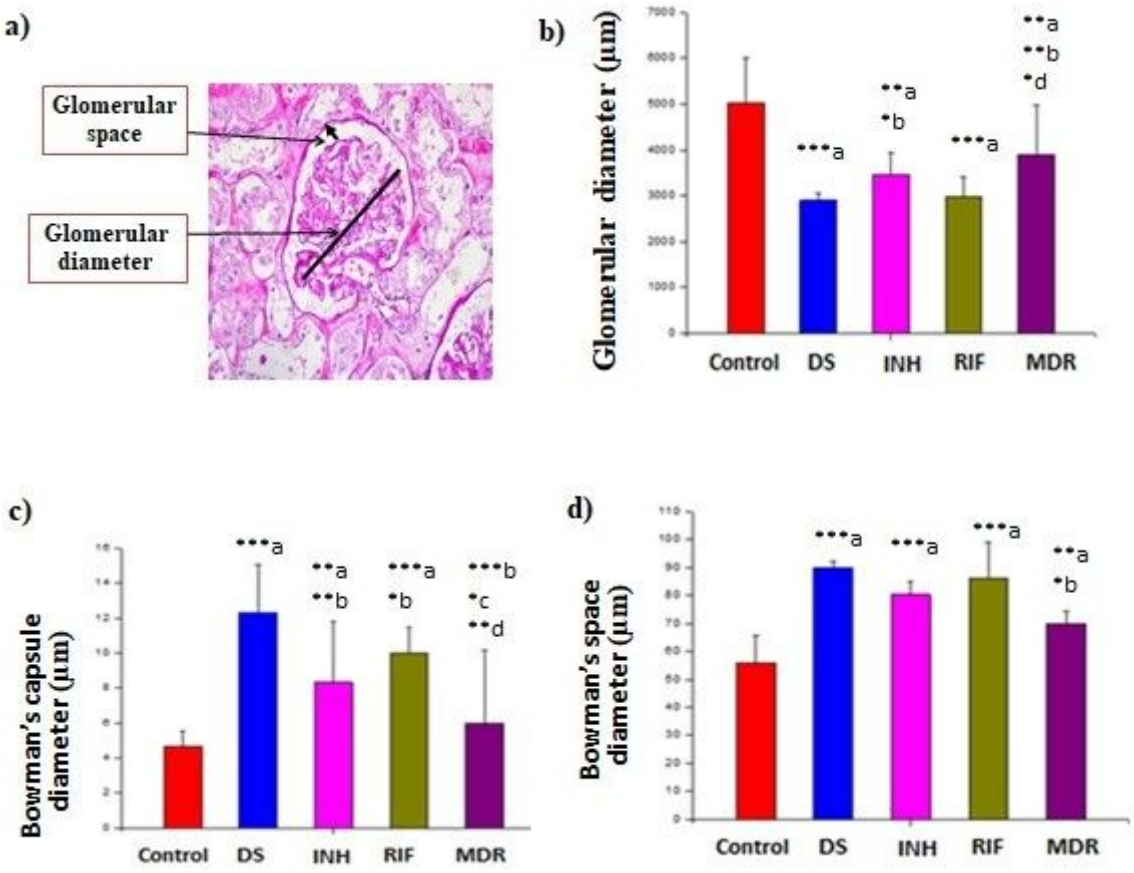

Figure 8

Glomerular space and Glomerular diameter **b)** Morphometric measurement of glomerular diameter of short-term animal groups **c** Quantitative analysis of Bowman's capsule diameter of short-term animal groups **d)** Quantitative analysis of Bowman's space diameter of short-term animal groups. Data were represented as mean ± SD, and one-way ANOVA followed by Tukey's post hoc test was done and p value less than 0.05 were taken statistically significant (***p≤0.001, **p≤0.01, *p≤0.05), ([a] compared to control, [b] compared to DS, [c] compared to INHR, [d] compared to RIFR).

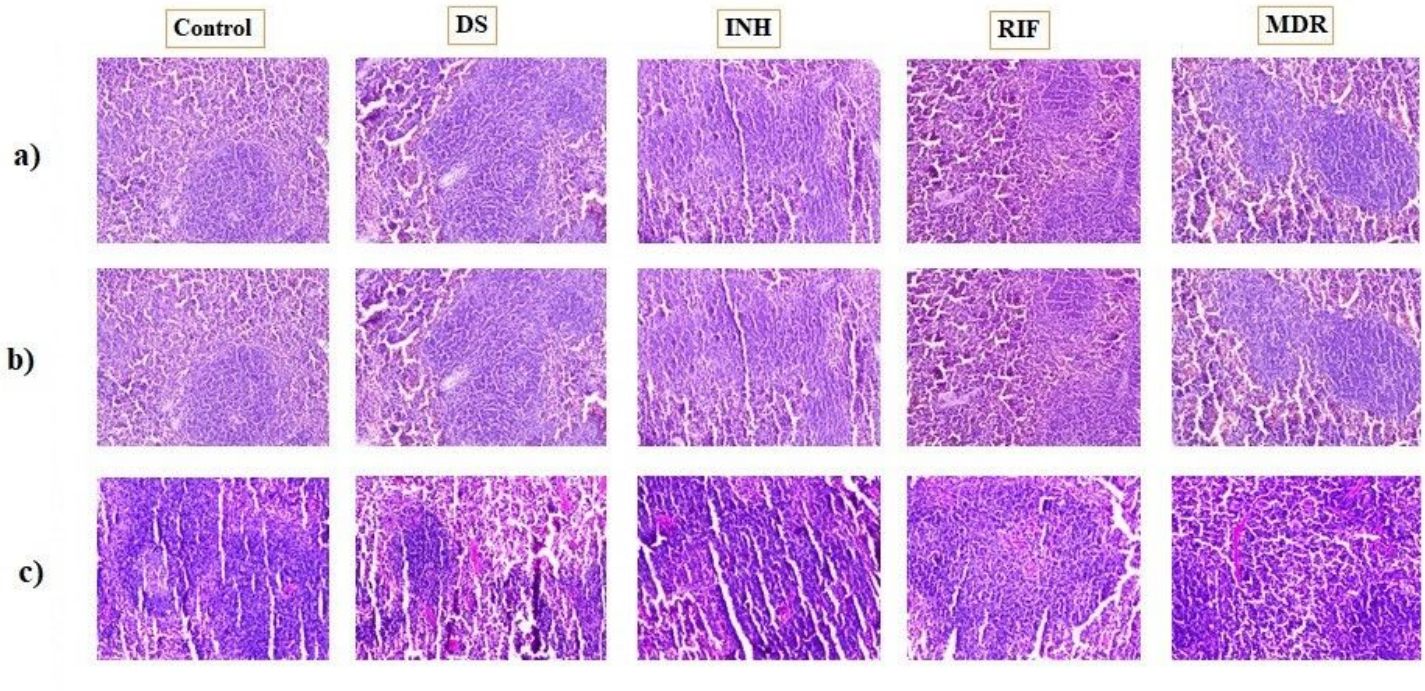

Figure 9

Histopathological examination of spleen tissue from short term animal groups stained with H&E (under 100X) **b)** Histopathological examination of spleen tissue from long term animal groups stained with H&E (under 400X) **c)** Histopathological examination of spleen tissue from short term animal groups stained with PAS (under 100X).

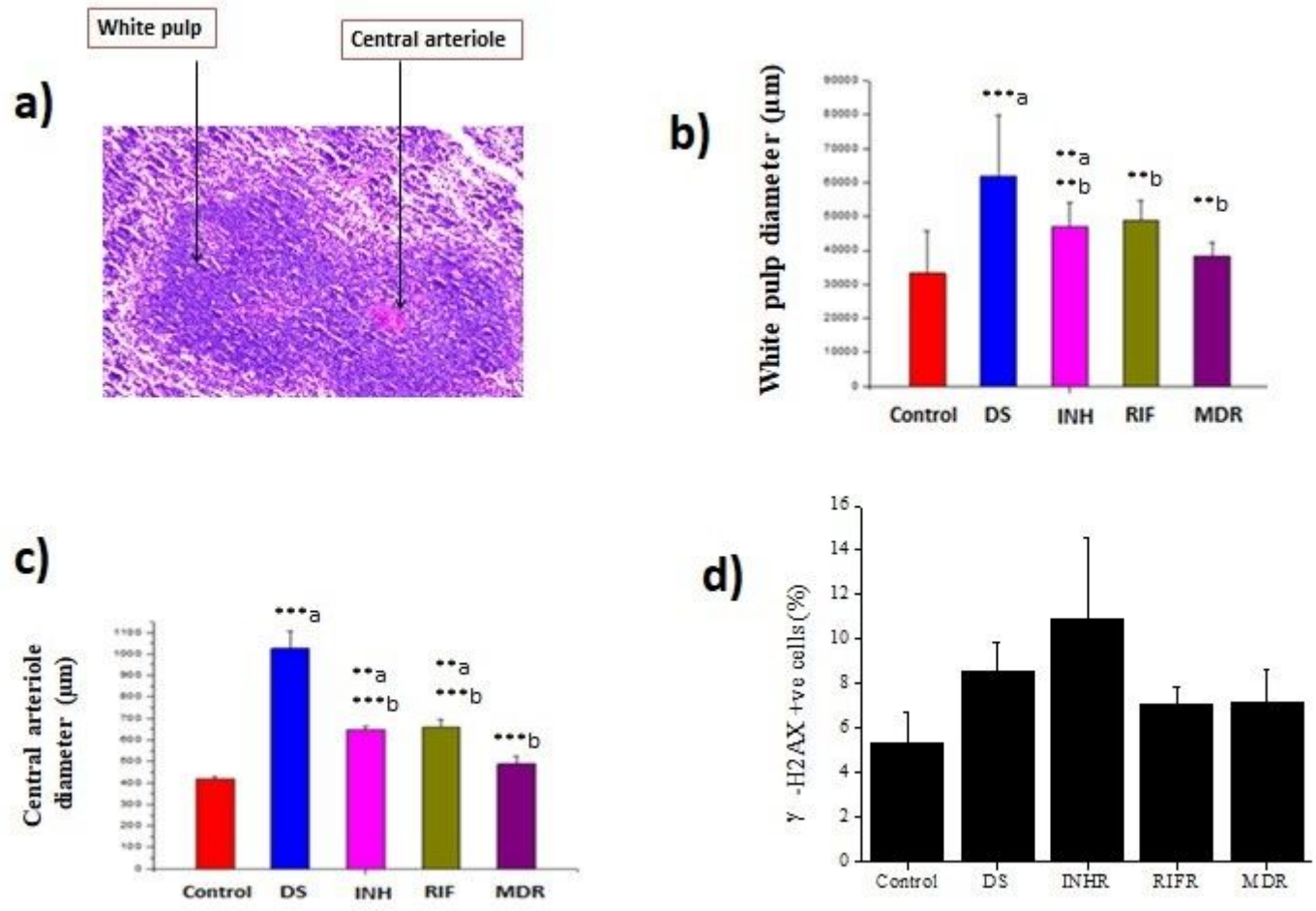

Figure 10

White pulp and central arteriole diameter b) Morphometric measurement of white pulp diameter of short-term animals group **c)** Quantitative analysis of central arteriole diameter of short-term animal groups d) Effect of ATDs used in DOTS schedule on DNA integrity of bone marrow cells in after the completion of treatment. Data were represented as mean ± SD, and one-way ANOVA followed by Tukey's post hoc test was done and p value less than 0.05 were taken statistically significant (***p≤0.001, **p≤0.01), ([a] compared to control, [b] compared to DS).

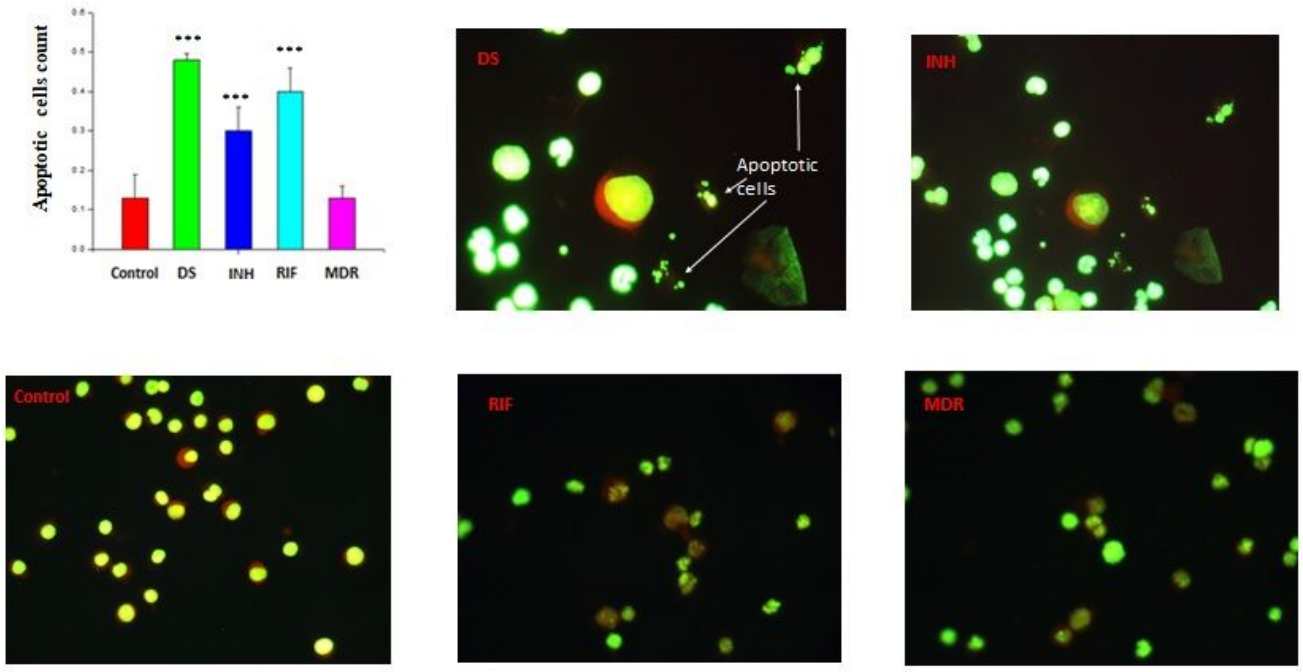

Figure 11

Apoptotic cells under fluorescence microscope stained with Acridine Orange (AO) of short-term animals group. Data were represented as mean ± SD, and one-way ANOVA followed by Tukey's post hoc test was done and p value less than 0.05 were taken statistically significant (***p≤0.001, **p≤0.01), ([a] compared to control, [b] compared to DS, [c] compared to INHR, [d] compared to RIFR).

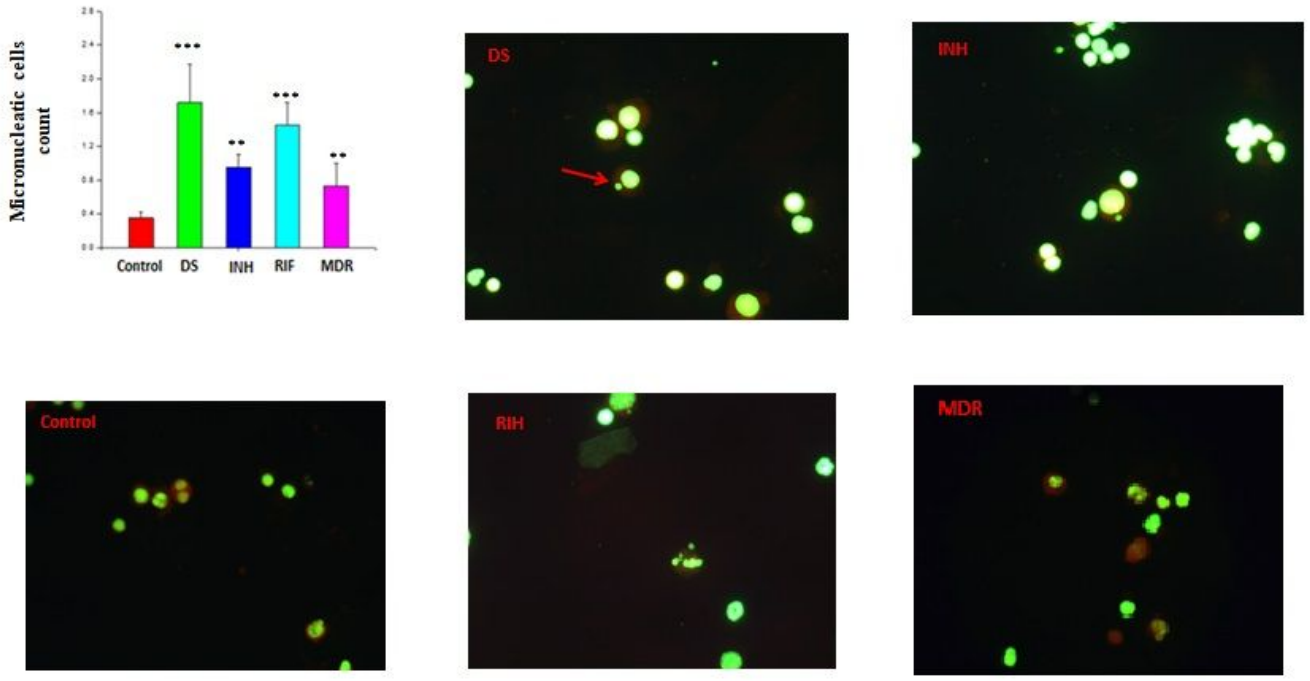

Figure 12

Micronucleatic cells under fluorescence microscope stained with AO of short-term animals group. Data were represented as mean ± SD, and one-way ANOVA followed by Tukey's post hoc test was done and p value less than 0.05 were taken statistically significant (***p≤0.001, **p≤0.01), ([a] compared to control, [b] compared to DS).