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Evaluation of polyherbal formulation against isoniazid and rifampicin induced oxidative stress in rat kidney

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The present study deals with oxidative stress in rat kidneys upon administration of Isoniazid (INH) and Rifampicin (RIF), and the attenuative effect of polyherbal formulation Heptoplus. Heptoplus is a polyherbal drug that contains phyllanthin as the active ingredient. Rats were intoxicated with INH and RIF and Heptoplus was orally fed to rats along with INH and RIF. Body weight, serum biochemical, antioxidant status, mitochondrial enzyme activity, lysosomal enzyme leakage and the histopathological impact were studied in the rat kidneys. Body and kidney weight of untreated rats showed deviation, but on treatment, they maintained their normal weight. Untreated rats showed altered serum urea and creatinine levels with deviated antioxidant status and enzymes leakages from lysosomes and decreased mitochondrial enzyme activity in kindey. But Heptoplus treatment allowed the rats to maintain their biochemical indices in blood and kidney at near normal level. Histopathological reports revealed INH- and RIF -induced oxidative stress was not in sufficient amount to incur any significant damage at the tissue level. Hence, both treated and untreated rat kidneys exhibits normal architecture. In conclusion, Heptoplus treatment protects the kidney of rats from the INH- and RIF- induced oxidative stress.

Keywords: Antioxidant, Heptoplus, Oxidative stress, Phyllanthin

IPC Code: Int Cl[21]: A61K 9/00, A61K 9/20, A61K 36/47, A61K 36/185, A61K 31/496, A61K 31/4409

Tuberculosis is the second largest mortality-causing disease in the world, with regard to a single infectious organism, despite the social economic development and medical advancements. In 2010, it consumed 1.4 million lives globally and in India alone, 330,000 deaths have been reported[1]. Isoniazid (INH) and Rifampicin (RIF) are the two prime chemotherapeutic drugs which are currently used for the treatment of tuberculosis. However, various adverse effects have been reported, which include hepatic dysfunction[2], kidney malfunction[3], hematological profile alteration[4] and neurotoxicity[5]. The reason provided for these adverse effects is oxidative stress caused by the reactive metabolite of INH, namely acetyl hydrazine and hydrazine (HYZ). Oxidative activation of these metabolites by cytochrome P450 generates a reactive oxygen species[6] which consumes the endogenous antioxidants, causing lipid peroxidation in the tissue. RIF is a strong inducer of CYP2E1, which aggravates the tissue toxicity by producing more reactive metabolite HYZ from INH, in both humans and rats[7]. Earlier reports revealed that mitochondrial dysfunction associated with glutathione depletion in the tissue was due to HYZ[8]. Since oxidative stress is an indispensable event of INH- and RIF- induced toxicity, in the present study the impact of INH- and RIF- induced oxidative stress in the kidneys of rat and the efficacy of Heptoplus were analyzed. Heptoplus is a polyherbal drug which contains phyllanthin as one of the active ingredients[9].

Materials and Methods

Chemicals and reagents

Isoniazid and rifampicin used in the study were obtained from Sigma Aldrich, USA. The rest of the chemicals and reagents were used in this study were of analytical grade from HiMedia Laboratories.

Heptoplus

Heptoplus (Tamil Nadu state-licensed drug, Lic. No 616), a polyherbal drug was purchased from Care and Cure Herbs Pvt Ltd, Chennai, India, in the form of a capsule. Each capsule has the following composition, described in Table 1.

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Table 1 — Composition of Heptoplus

S. No.	Herbal plants	Parts of the plant used	Quantity (mg)/capsule
1	*Phyllanthus amarus* Schumach. & Thonn.	Whole plant	100
2	*Eclipta alba* Linn Hassk.	Leaves	50
3	*Tephrosia purpurea* L. Pers.	Leaves	30
4	*Curcuma longa* Linn	Rhizome	30
5	*Picrorhiza kurroa* Royle ex Benth.	Root	20
6	*Withania somnifera* Linn.	Root	100
7	*Pinus succinifera* Linn.	Amber	37.50
8	*Pistacia lentiscus* Linn.	Resinous exudate	25.00
9	*Orchis masculaL.*	Endosperm	25.00
10	*Cycas circinalis* L.	Flower Male	62.50

Maintenance of animals

Male Sprague Dawley rats (150 to 200 g) used in this study were procured from Sri Ramachandra Medical College, Chennai, India. The entire study protocol was approved by the Institutional Animal Ethics Committee, New Delhi, India, (IACE/ XXII/SRU/168/2011). The rats were accommodated individually in well-contained ventilated polypropylene cages.. A 12- h light & 12-h dark artificial photoperiod, 22±3° room temperature and 50%–70% relative humidity were maintained in the room. Rats were allowed free access to the pelleted feed (Nutrilab rodent, Tetragon Chemie Pvt Ltd., India) and reverse osmosis purified water (Rios, USA) was provided.

Experimental assay protocol

Rats were grouped into four, with 6 rats in each group. The drugs were administered orally one time daily for a period of 30 days.

Group I: Rats received CMC (vehicle), served as control

Group II: Rats were intoxicated with 50 mg/kg of each RIF and INH (toxic control)[10]

Group III: Rats treated with 100 mg/kg of heptoplus (HP) along with 50 mg/kg each of RIF and INH.

Group IV: Rats treated with heptoplus (HP control) alone 100 mg/kg b.w.

Biochemical assay

Blood samples were collected from the retro orbital venous plexus of rats on 15th and 30th day of experimental periods.The serum was separated and used for the quantification of urea and creatinine as per Merk India biochemical kit method.

Antioxidant assay

After the experimental period, the rats were decapitated under aseptic conditions and the kidneys were surgically removed and blotted. After that, tissue was homogenized with phosphate buffer pH 7.5 and centrifuged (3000 g for 10 mts). The supernatant collected was used for the following the assay of Catalases (CAT)[11], Superoxide dismutase (SOD)[12], Glutathione (GSH)[13] and lipid peroxidation (LPO)[14].

Assay for mitochondrial enzyme activity

The mitochondria were isolated from the rat kidneys[15]. After isolation the following assays were carried out. Isocitrate Dehydrogenase[16], α-Keto Glutarate Dehydrogenase[17], NADH Dehydrogenase[18] and Malate Dehydrogenase[19].

Assay for Lysosomal enzymes

The tissue lysosomal fraction was prepared based on the method by Plummer[20]. After the isolation of lysosomes from the kidney of rats, β-Galactosidase[21] and Cathepsin D[22] assays were carried out.

Histopathological analysis

Histopathological examination of tissue was performed as per the method of Humason 1979[23].

Data analysis

The numerical data were expressed in the form of Mean ± SEM. The statistical significance of the numerical data was analyzed by using the one-way analysis of variance (ANOVA), then followed by Turkey-Kramer multiple comparison tests, using Graph pad prism software package for Windows (Version 5). The p value<0.05 was considered as significant with respect to group I control rats.

Results

Effect of heptoplus on kidney and the body weight of rats

The body and kidney weight of rats were taken in to consideration in order to evaluate the efficacy of the herbal drug. The data obtained have been shown in Figure 1 and 2. Observation of intoxicated rats shows significant decrease (Fig. 1) in the body weight ($p<0.05$) in 14th, 21st, 28th and 30th days of study period, whereas heptoplus- fed rats shows no significant decrease in body weight, when compared to control. A non-significant decrease in kidney weight (Fig. 2) was observed in the untreated rats.

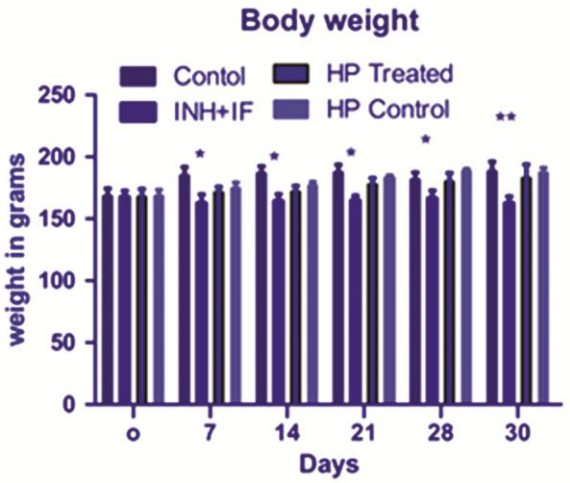

Fig. 1 — Effect of Heptoplus on body weight of rats: N=6, all value expressed in mean± SEM, p≤ 0.05* consider as significant compared to group I (control) .

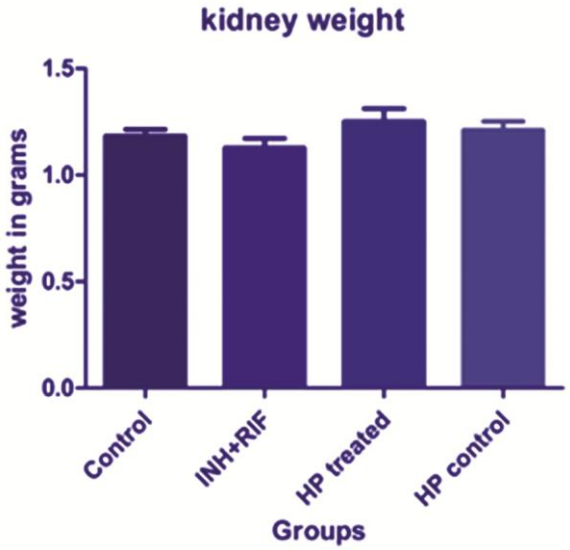

Fig. 2 — The impact of Heptoplus on rats' kidney weight: N=6, all value expressed in mean± SEM, p≤ 0.05* consider as significant compared to group I (control)

Heptoplus-treated rats have not shown any deviation in their kidney weight when compared to control ones.

Effect of heptoplus on serum urea and creatinine level

The serum creatinine and urea level of rats on 15th and 30th day of study period are represented in the Figure 3 and 4. The intoxicated rats in group II showed a significant increase (≤ 0.05) of serum creatinine and urea levels in both the days of screening period, when compared to group I control rats. However, Heptoplus- treated rats in group III showed near normal-levels of serum urea and

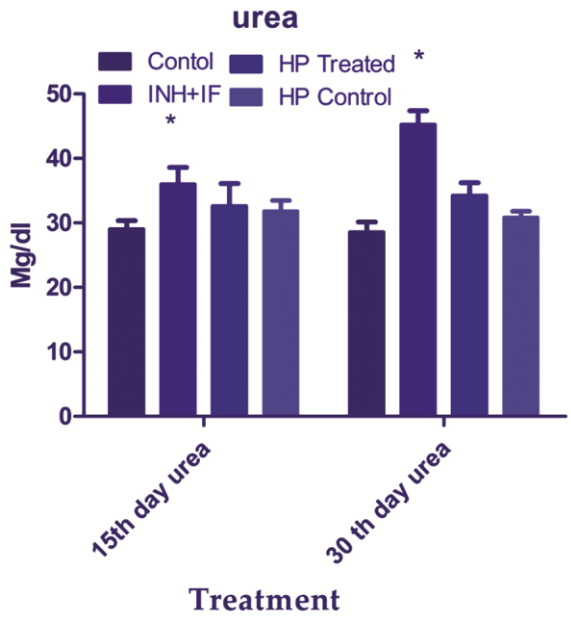

Fig. 3 — Effect of Heptoplus on serum urea levels: N=6, all value expressed in mean± SEM, p≤0.05* consider as significant compared to group I (control) .

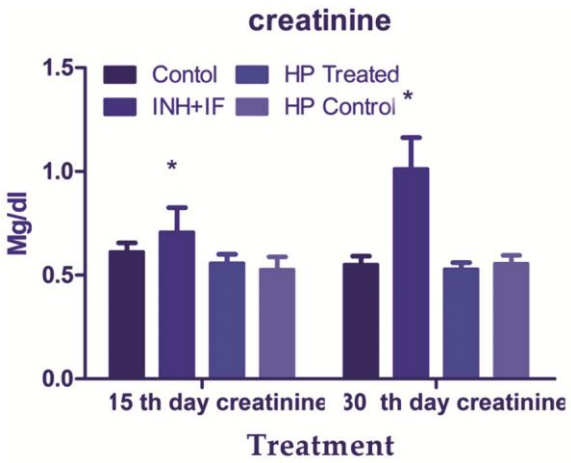

Fig. 4 — Effect of Heptoplus on serum creatinine levels: N=6, all value expressed in mean± SEM, p≤0.05* consider as significant compared to group I (control) .

creatinine on comparsion with the group II rats. Heptoplus control rats in group VI also showed a near-normal level of serum creatinine and urea.

Effect of Heptoplus on antioxidant status

Antioxidants status in the kidney of rats was studied for Heptoplus-treated and untreated groups. The data obtained are presented in Table 2. The kidneys of intoxicated rats showed significant decrease in GSH ($p<0.05$) content. However, Heptoplus-fed rats showed normal GSH content in kidney. A sharp decline ($p<0.05$) of CAT activity was

Table 2 — Antioxidant activity of Heptoplus on isoniazid and rifampicin induced oxidative stress in the rat kidney.

Parameters /groups	Group I (Control)	Group II (toxic control)	Group III (Heptoplus treated)	Group VI (HP control)
GSH (µM GSH/ mg protein.)	1.67±0.01	1.02±0.81*	1.57±0.13	1.62±0.15
CAT (µm of H_20_2 consumed/min/mg protein)	0.54±0.51	0.32±0.04*	0.54±0.06	0.56±0.05
SOD (U/mg protein)	27.31±2.36	23.10±3.38	26.25±0.61	26.83±2.46
LPO (nM MDA formed/ h/ mg protein)	7.56±0.90	9.18±0.54*	7.27±0.7	7.29±0.37

N=6, The numerical values are expressed in form of mean± SEM, p≤0.05* consider as significant compared to group I rats (control)

Table 3 — Effect of Heptoplus on kidney mitochondrial dehydrogenase activity.

Mitochondrial enzymes activity	Group I (Control)	Group II (INH + RIF)	Group III (HP Treated)	Group VI (HP control)
Isocitrate dehydrogenase (α-KG liberated / min / mg of total protein)	3.12±0.02	2.03±0.8*	3.10±0.51	3.00±.026
α-keto glutarate dehydrogenase (potassium ferrocyanide liberated/ mg protein)	37.03±2.62	30.30±3.1	39.43±3.66	38.00±3.4
NADH dehydrogenase (n mol NADH oxidized/mg of protein)	1.37±0.81	0.97±0.90	1.48±0.70	1.40±0.39
Malate dehydrogenase (µmol NADH oxidized/mg of protein)	18.15±2.2	15.30±1.10	18.80±1.43	17.78±1.35

N=6, The numerical values are expressed in form of mean± SEM, p≤ 0.05* consider as significant compared to group I rats (control)

observed in the kidneys of rats in group II, when compared to group I (normal). But on Heptoplus treatment, rats kidneys showed normal CAT activity. A decline of SOD activity was observed in the kidneys of rats on INH and RIF treatment. On Heptoplus treatment, SOD was restored in the kidneys of rats. LPO is an index of free radical generation. Intoxicated rats showed a significant increase ($p<0.05$) of LPO when compared to group I control rats. But on Heptoplus treatment,the LPO levels were near normal.

Effect of Heptoplus on mitochondrial dehydrogenase activity

Mitochondrial dehydrogenase activity in the kidneys of rats were studied, in order to ascertain the impact of INH and RIF and the efficacy of Heptoplus on mitochondria; the data obtained are shown in Table 3. Kidneys of intoxicated rats exhibits a sharp decrease ($p<0.05$) in the mitochondrial IDH activity, when compared to group I control rats. But on treatment with Heptoplus, the mitochondria IDH activity was completely restored to normal. Decreased α-KGDH activity was observed in the kidney of rats on treatment with INH and RIF. However, on Heptoplus treatment the α-KGDH activity was found to be normal. A slight decrease in the NADH dehydrogenase and MDH activity in the kidney of intoxicated rats was observed. However, on Heptoplus treatment, the activity of NADH dehydrogenase and MDH activity was found to be normal in kidney of rats.

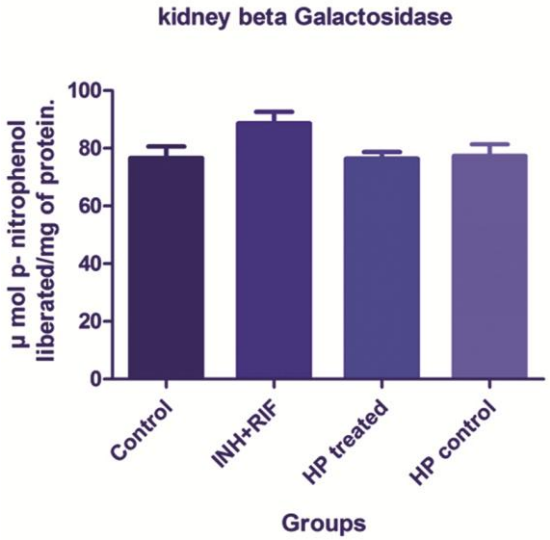

Fig. 5 — Effect of Heptoplus on β-galactosidase activity: N=6, all value expressed in mean± SEM p≤0.05* consider as significant compared to group I (control) .

Effect of Heptoplus on lysosomal integrity

The lysosomal integrity in the kidney of rats was studied, based on the leakages of β -galactosidase and cathepsin D in the lysosomal fraction, the data obtained are shown in Figures 5 and 6. The lysososmal fraction from the kidney of INH- and RIF- treated rats did not exhibited significant increase of β -galactosidase and cathepsin D activity, when compared to control rats in group I. Heptoplus-treated rats also exhibited normal β -galactosidase and cathepsin D activity, at par with control rats in group I.

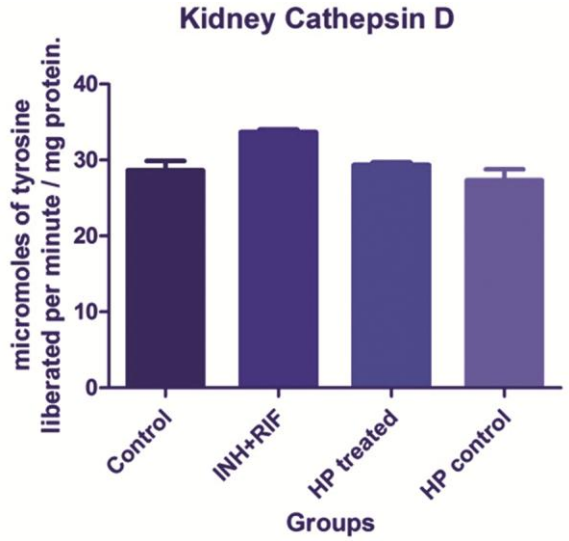

Fig. 6 — Effect of Heptoplus on cathepsin D activity: N=6, all value expressed in mean± SEM, p≤0.05* consider as significant compared to group I (control) .

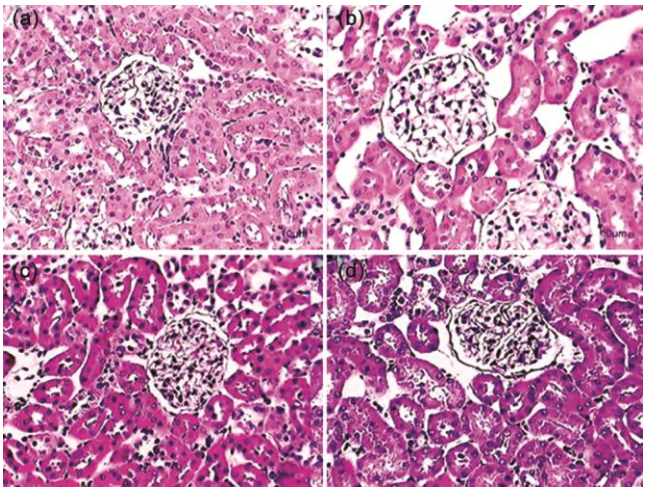

Fig. 7 — Histopathological status of rat kidneys with the presence of Heptoplus: 10x photomicrographic images of Kidney sections of rats exhibiting normal glomeruli, tubules and vessels. There is no apparent modification of kidney architecture in any one of the groups. Both intoxicated (group II) and Heptoplus treated rats were showed a normal architecture of kidneys. Heptoplus control rats in group IV also showed a normal architecture of the kidney. A) control (B) INH and RIF treated (C) Heptoplus treated and (D) Heptoplus control.

Histopathological analysis of kidney of rats

Histopathological analysis of rat kidneys is shown in Figure 7. Although INH- and RIF- treated rats showed abnormal index of biochemical profiles, but on histopathological analysis, normal architecture was seen. Heptoplus-treated rat kidneys also showed a normal architecture. From this analysis, it is inferred that INH and RIF administration does not create any apparent modification in the kindeys of rats.

Discussion

The present study dealt with INH- and RIF-induced oxidative stress and the efficacy of Heptoplus in kidneys of rats. The word "oxidative insult" refers to a gross deviation between reactive oxygen species (ROS) production and antioxidant defense[24]. Earlier studies reported that INH and RIF administration may cause the depletion of reserve antioxidants in tissue, which is one of the key driving forces for pathogenesis[8]. ROS and oxidative stress play a vital role in drug-induced renal damage. Although INH and RIF are known for their hepatotoxicity due to release of adverse free radicals from their toxic metabolites, the accumulated free radicals from these also can cause the renal tubular damage by consuming their reserve antioxidant status[25]. Body weight index is one of the vital parameters for the evaluation of adverse effects in any drug[26]. In the present study, declined body weight trend in untreated rats indicates the sickness and severity, which may be the outcome of oxidative stress impact. But with heptoplus treatment, rats maintained their body weight, which is an impact of healthiness. The sign of non-significant alternation in the kidney weight of untreated rats, indicates the least impact of INH and RIF exposure.

Significant deviation of renal biochemical makers such as serum urea and creatinine levels shows the impairment of renal function due to course of INH and RIF. However, with Heptoplus application, there is no such renal malfunction was found.

Since oxidative stress is one the causative mechanisms of impairment of renal damage,in the present study, GSH content in the kidneys of rats was assessed. GSH is a crucial endogenous antioxidant, which scavenges reactive oxygen intermediates (ROS) and maintains the intracellular redox balance to protect the tissue from oxidative insult[27]. The homeostatic decrease of GSH contents leads to the development of the further course of damage to the tissue, which is induced by toxins[28]. Earlier studies indicated that INH is highly reactive with the sulfhydryl group of GSH, which results in the depletion of GSH contents in the liver cell[8]. In the present study reduction of GSH content in the kidneys of intoxicated rats, may be due to the consequence of maximum utilization for ROS detoxification and /or oxidation of their sulfhydryl groups. However, Heptoplus-treated rats were able to maintain the intracellular redox balance by sustaining the GSH content in the tissue. The sustaining of GSH content on Heptoplus treatment is due to the presence of

active ingredient phyllanthin, which may restrict the oxidation of sulfhydryl groups and reduced the utilization of GSH for ROS detoxification.

CAT and SOD are the prime enzymatic antioxidant systems present in the cells which scavenge the free radicals and give protection against oxidative stress. SOD catalyses the conversion of superoxide anion in to H_2O_2, whereas CAT is involved in the detoxification of H_2O_2 formed either from the free radicals or by SOD on removal of super oxide anion. Therefore, any reduction in the activity of CAT and SOD may occur due to assimilation of superoxide anion[29]. The excessive formation of superoxide anion may inactivate SOD and CAT[30]. In the current observation, reduction in the activity of CAT and SOD in tissue indicates assimilation of free radicals from INH-and RIF-administration. The restoration of normal CAT and SOD activity in the kidneys of rats indicates dissimilation of superoxide anion on Heptoplus treatment. The dissimilation of superoxide anion might be due to the presence of antioxidants in the Heptoplus, which could have inhibited the generation of free radicals from INH and RIF. LPO index is used to measure the damage acquired by the tissue as a consequence of free radical generation[31]. Elevation of hepatic LPO is always accompanied with a sharp decline in the intracellular antioxidant system, considered as a marker of enhanced oxidative insult in INH-and RIF-induced hepatotoxicity[32]. In corroboration with the results obtained by Ali,[32] in the present study also, elevated level of LPO with a concomitant decline of intracellular antioxidant was observed in the kidney of intoxicated rats, which indicated the development of oxidative insult. In Heptoplus treatment group, the LPO levels were under control and with a concomitant increase of intracellular antioxidants. The decline of LPO level on Heptoplus treatment might be due to its free radical scavenging property.

Mitochondrial function in the kidney of rats was taken into consideration to study the impact of oxidative insult on administration of INH and RIF. Mitochondrial dysfunction and ROS generation are indispensable events of INH- and RIF- induced toxicity[8]. Excessive generation of ROS inhibits the activities of TCA cycle enzymes and NADH dehydrogenase. This eventually leads to inefficient electron transport and causes oxidative damage to the mitochondria. Inhibition of these enzymes may tamper the mitochondrial substrate oxidation reactions, which results in decreased ATP production[33]. Previous studies have shown that the administration of INH- and RIF- decreases the activity of specific dehydrogenases[34]. In accordance with Prabakan *et al*[34], mitochondrial dehydrogenases activity was reduced on INH-and RIF- treatment, which may be due to the outcome of excessive ROS generation. But with Heptoplus treatment, normal mitochondrial dehydrogenases activity was witnessed in the kidneys of rats, which indicated curtailment of ROS generation. Therefore, Heptoplus treatment could provide the necessary antioxidants like phyllanthin to the tissue to curtail the excessive ROS generation from INH and RIF, thereby to retaining the mitochondrial dehydrogenases activity.

Lysosomes are distinct cell organelles, which contain a variety of enzymes. The magnitude of the enzyme leakage from the lysosomes, determines the type of cell death. The assessment of enzyme leakage is necessary because, during oxidative insult, reactive radicals may interact with the lipid bilayer of various organelles, including lysosomes, as a result, destabilization of lysosomal membrane occurrs and leads to lysosomal rupture. Any compromise in the lysosomal membrane integrity may lead to an unacceptable elevation of enzymes in the intra- and extra-cellular space, which induces apoptosis process[35]. The increase of β-galactosidase and cathepsin D activity in the lysosomal fraction of the untreated rats indicates the presences of lysosomal rupture in the kidney. The rupture in the kidney lysosomal membrane of the intoxicated rats may be the impact of an oxidative insult. However, on Heptoplus treatment, the undesirable enzymatic activity was not inferred in the lysosomal fraction, this could be possible only if the kidney lysosomal membrane was intact.

The impact of oxidative stress in the course of tissue damage was analyzed. Histopathological report reveals intoxicated rat kidneys exhibit a normal architecture. This may be due to that the toxicant-induced oxidative stress is not sufficient to produce kidney damage, though it may get reflected in the biochemical parameters.

Conclusion

From this study, it can be concluded that, Heptoplus supplement, enables the rats to maintain their intracellular antioxidant levels to curtail the development of oxidative stress in the kidney on

administration of INH and RIF. Similarly, the impact of oxidative stress on administration of INH and RIF was not in a sufficient amount to induce any significant damage at the tissue level.

Confilct of interest

The authors declare that there is no conflict of interest.

Author (s) contribution

Sankar M carried and executed the entire work with guidance of J R and D S.

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