Hepatotoxic Assessment of Co-treatment with Isoniazid and Efavirenz in Albino Rats

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Abstract
This study comparatively investigated the toxicological effects of treatment with efavirenz, isoniazid and efavirenz-isoniazid (EFV- INH) combination on liver function parameters and histology of adult male albino rats. Animals used in this study were divided into five (5) groups A-E of sixteen (16) animals each. Animals in group A (placebo control) were treated with water while animals in group B (solvent control) were treated with arachis oil orally. Animals in groups C-E were treated orally with 15mg/kg of INH, 10mg/kg of EFV and a combination of INH - EFV for 2-8 weeks respectively. At the end of drug therapy, serum was extracted from centrifuged blood sample and evaluated for alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total and conjugated bilirubin. Animals were sacrificed and liver was harvested, weighed and evaluated for histopathological changes. Effects produced by co-treatment with EFV-INH combination showed vascular congestion, inflammation of parenchyma and hepatocytes degeneration. These results show that co-treatment with EFV-INH in patients with human immunodeficiency virus/tuberculosis co-infection may not be associated with synergistic hepatotoxicity.

Keywords Liver, Toxicity, Interaction, Isoniazid, Efavirenz, Rats

1. Introduction

Human immunodeficiency virus (HIV) pandemic poses major threat to the socio-economic and psychological welfare of HIV infected people with decrease life expectancy. But the life expectancy of HIV-positive subjects has dramatically improved with the use of triple antiretroviral drug combinations [1]. Despite increase in life expectancy of HIV infected patients, toxicological effects of antiretroviral therapy have become a limiting cause of benefit in a substantial proportion of patients. Hepatotoxicity is one of the limiting toxicological effects that has been reported by many centers’ in developed world and is now recognized as a major cause of morbidity and mortality in patients receiving antiretroviral treatment [2]. Among antiretroviral drugs, the use of Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs), as common component of antiretroviral therapy, has been associated with the development of hepatotoxicity in a large number of studies [3, 4]. Efavirenz is one of the NNRTIs that have been implicated in hepatotoxicity in HIV-positive patients, especially where co-infection with hepatitis viruses is present [5-7]. Several clinical trials have provided information on the frequency of severe hepatotoxicity of grade 3 or above due to efavirenz [8]. Treatment with efavirenz containing antiretroviral regimens could be associated with fulminant liver failure which may lead to death [9].

Tuberculosis (TB) remains one of the most important communicable diseases in the world. The world health organization estimates that one-third of the world’s population is infected with mycobacterium tuberculosis. Anti-tuberculosis drugs commonly used for drug-susceptible tuberculosis include isoniazid, rifampicin and pyrazinamide. The use of anti-tuberculosis drugs has been characterized by incidence of hepatotoxicity [10, 11]. Higher incidence of hepatotoxicity has been commonly reported with the use of isoniazid (INH) containing anti-tuberculosis regimens [12, 13]. Isoniazid could be associated with progressive hepatic failure characterized by alteration in liver histology that may require liver transplantation [14]. Histopathological changes induced by isoniazid therapy were reported to be characterized by ballooning degeneration, and focal hepatocytes necrosis with minimal cholestasis [15]. Also elevations in transaminases have been reported with isoniazid therapy [16].

There are well established epidemiological and biological
synergies between HIV and TB, influencing the distribution, progression and outcomes of both infections [17, 18]. The established relationship between HIV and TB infections has necessitates co-therapy with antiretroviral drugs and anti-tuberculosis drugs. These include cases of co-therapy with isoniazid and efavirenz containing anti-tuberculosis regimens [19]. Efavirenz and isoniazid have been individually implicated in hepatotoxicity [20, 21] so co-therapy with these drugs may cause substantial disruption in the anatomy of the liver which may result in severe alteration in its metabolic roles and physiological functions. This study therefore, comparatively evaluated the toxicological effects of treatment with isoniazid, efavirenz and isoniazid-efavirenz combination on liver function parameters and histology of male albino rats.

2. Materials and Methods

Animals

Eighty (80) adult male albino rats of average weight 320±5 g were used in this study. The rats were obtained from the animal house of the Department of Pharmacology and Toxicology, Madonna University, Elele, Rivers State. The rats were allowed to acclimatize for 14 days with free access to food and water ad libitum.

Ethical Considerations

All animals used in this study were handled in accordance with the international, national and institutional guidelines for care and use of laboratory animals in biomedical research as promulgated by the Canadian Council of Animal Care. [22]

Drugs

Isoniazid used in this study was manufactured by Mancare Pharmaceuticals, India while efavirenz was manufactured by Mylan Laboratory India. Isoniazid 15mg/kg and efavirenz 10mg/kg were used in this study [22-25]. Efavirenz and isoniazid tablets were crushed and suspended in arachis oil. All other chemicals used in this study were of analytical grade.

Drug Administration

Group A: This served as the placebo control and contained sixteen (16) rats which were treated orally with water.

Group B: This served as the solvent control and contained sixteen (16) rats which were treated with arachis oil orally.

Group C: This group contained sixteen (16) rats which were further divided into four subgroups of four animals each and were treated with 15mg/kg of isoniazid orally for 2-8 weeks respectively.

Group D: This group contained sixteen (16) rats which were further divided into four subgroups of four animals each and were treated with 10mg/kg of efavirenz orally for 2-8 weeks respectively.

Group E: This group contained sixteen (16) rats which were further divided into four subgroups of four animals each and were treated with combined doses of 15mg/kg of isoniazid and 10mg/kg of efavirenz orally for 2-8 weeks respectively.

Collection of Sample for Analysis

At the end of 2, 4, 6 and 8 weeks of treatment respectively, under diethyl ether anesthesia, 2ml of blood was collected directly via cardiac puncture into sterile sample containers. The sample was allowed to clot and centrifuged at 1000 rpm for 5mins and serum separated for biochemical analysis. Liver was collected through dissection, weighed and evaluated for histopathological changes.

Evaluation of Serum Liver Function Parameters

Estimation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was done using Reitman-Frankel method 1975 [26]. Estimation of alkaline phosphatase (ALP) was performed using King and Kind Method, 1954 [27]. Total bilirubin (TB) and conjugated Bilirubin (CB) were evaluated as reported by Ogbuieh et al., 2014. [28]

Histopathological Analysis

For histopathological examinations, pieces of the liver were fixed in 10% neutral buffered formalin, dehydrated in ascending series of ethanol, cleared in methyl benzoate and embedded in paraffin wax. Paraffin sections of 5 microns in thickness were prepared and stained with hematoxylin and eosin (H and E) and examined with the aid of a microscope for histopathological changes.

Statistical Analysis

This was done using graph pad prism 5 statistical package and ANOVA for comparison of the means of the various groups. Results are expressed as Mean± standard error of mean (S.E.M). Statistical significance was set at \( p<0.05 \)

3. Results

Effects on Absolute Liver Weight and Serum Alanine Aminotransferase (ALT)

Absolute liver weight of animals treated with INH, EFV and INH–EFV combination did not differ significantly \( (p>0.05) \) from that of the control. In this study, treatment with INH for 2-8 weeks time-dependently increased serum ALT levels with significant \( (p<0.05) \) increases to 41.2±0.48 and 49.5±0.22 u/l observed at weeks 6 and 8 respectively when compared to the control. Also, treatment with EFV produced time-dependent increases in serum ALT levels with significant \( (p<0.05) \) increase observed at week 8 when compared to the control. It was also noted that co-treatment with INH–EFV for 2-8 weeks produced time-dependent increases in serum ALT levels. These increases were insignificant \( (p>0.05) \) when compared to increases produced by individual doses of INH and EFV [Table 1 and 2].
Effects on Serum Aspartate Aminotransferase (AST)

Furthermore, observations in this study show time-dependent increases in serum AST levels in INH treated animals with significant \( p<0.05 \) increases which represent 42% and 61% observed at weeks 6 and 8 respectively when compared to the control. Treatment with EFV also produced time-dependent increases in serum AST levels with observed significance \( p<0.05 \) at week 8 when compared to produced by individual doses of EFV and INH [Table 3].

Effects on Serum Alkaline Phosphatase (ALP)

Time-dependent increases in serum ALP levels were observed in INH treated animals with significant \( p<0.05 \) increases to 84.9±2.10 and 92.5±2.24 u/l observed at weeks 6 and 8 respectively when compared to the control. EFV treated animals also showed time-dependent increases in serum ALP levels with observed significance \( p<0.05 \) at week 8 when compared to control. Furthermore, treatment with a combination of INH -EFV produced insignificant \( p<0.05 \) time-dependent increases in serum ALP levels when compared to treatment using individual doses of INH and EFV [Table 4].

Effects on conjugated bilirubin and total bilirubin

Observations in this study show that treatment with individual doses of INH and EFV did not produce significant \( p>0.05 \) effects on serum conjugated and total bilirubin when compared to the control. Also, treatment with a combination of INH- EFV did not produce significant \( p>0.05 \) effects on serum conjugated and total bilirubin levels when compared to effects produced by individual doses of INH and EFV [Table 5 and 6].

Effects on Liver Histology

Histological examination of liver of animals treated with individual doses INH and EFV and a combination of INH- EFV showed vascular congestion, inflammation of the parenchyma and degeneration of hepatocytes [Figure 2- 4].

### Table 1. Effects of treatment with isoniazid and efavirenz on absolute liver weight (g) of male albino rats

| Dose     | WK2 | WK4 | WK6 | WK8 |
|----------|-----|-----|-----|-----|
| Control  | 3.22±0.04 | 3.29±0.03 | 3.30±0.04 | 3.21±0.03 |
| INH 15mg/kg | 3.30±0.05 | 3.27±0.01 | 3.24±0.06 | 3.26±0.04 |
| EFV 10mg/kg | 3.22±0.30 | 3.30±0.05 | 3.37±0.02 | 3.39±0.09 |
| INH/EFV | 3.35±3.12 | 3.47±0.03 | 3.31±0.08 | 3.21±0.05 |

INH: Isoniazid. EFV: Efavirenz. Results are expressed as mean ± SEM. n=4

### Table 2. Effects of treatment with isoniazid and efavirenz on serum aspartate aminotransferase (U/L) of male albino rats

| Dose     | WK2 | WK4 | WK6 | WK8 |
|----------|-----|-----|-----|-----|
| Control  | 27.6±2.21 | 28.2±3.13 | 28.9±2.14 | 27.6±0.14 |
| INH 15mg/kg | 29.5±1.15 | 33.1±2.43 | 41.2±0.48 | 49.5±0.22* |
| EFV 10mg/kg | 28.6±0.65 | 30.75±1.14 | 33.5±2.51 | 40.7±2.23* |
| INH/EFV | 31.2±2.06 | 33.95±1.48 | 43.1±2.38* | 50.3±0.31* |

INH: Isoniazid. EFV: Efavirenz. Results are expressed as mean ± SEM. n=4. The superscript (*) means significant difference with respect to the control at \( p<0.05 \). (ANOVA).

### Table 3. Effects of treatment with isoniazid and efavirenz on serum aspartate aminotransferase (U/L) of male albino rats

| Dose     | WK2 | WK4 | WK6 | WK8 |
|----------|-----|-----|-----|-----|
| Control  | 43.0±3.12 | 42.9±1.26 | 43.1±2.13 | 42.7±1.15 |
| INH 15mg/kg | 45.5±2.30 | 50.1±2.12 | 61.2±1.34* | 68.5±1.13* |
| EFV 10mg/kg | 42.3±1.23 | 46.75±2.15 | 50.5±0.31 | 59.7±1.21* |
| INH/EFV | 49.2±2.00 | 51.0±1.22 | 63.1±0.18* | 69.3±3.42* |

INH: Isoniazid. EFV: Efavirenz. Results are expressed as mean ± SEM. The superscript (*) means significant difference with respect to the control at \( p<0.05 \) (ANOVA). n=4

### Table 4. Effects of treatment with isoniazid and efavirenz on serum alkaline phosphatase (U/L) of male albino rats

| Dose     | WK2 | WK4 | WK6 | WK8 |
|----------|-----|-----|-----|-----|
| Control  | 60.9±1.22 | 61.5±2.12 | 60.7±0.23 | 61.9±2.14 |
| INH 15mg/kg | 65.5±1.20 | 72.3±1.20 | 84.9±2.10* | 92.5±2.24* |
| EFV 10mg/kg | 62.4±2.10 | 67.5±0.32 | 72.3±1.20 | 87.2±2.13* |
| INH/EFV | 67.3±3.10 | 72.9±1.31 | 86.1±2.16* | 94.5±1.22* |

INH: Isoniazid. EFV: Efavirenz. Results are expressed as mean ± SEM. n=4. The superscript (*) means significant difference with respect to the control at \( p<0.05 \). (ANOVA).

### Table 5. Effects of treatment with isoniazid and efavirenz on serum conjugated bilirubin (mg/dl) of male albino rats

| Dose     | WK2 | WK4 | WK6 | WK8 |
|----------|-----|-----|-----|-----|
| Control  | 0.46±0.07 | 0.48±0.02 | 0.44±0.03 | 0.47±0.08 |
| INH 15mg/kg | 0.47±0.01 | 0.48±0.06 | 0.46±0.01 | 0.50±0.04 |
| EFV 10mg/kg | 0.45±0.03 | 0.44±0.08 | 0.47±0.04 | 0.43±0.09 |
| INH/EFV | 0.47±0.06 | 0.49±0.07 | 0.45±0.06 | 0.46±0.01 |

INH: Isoniazid. EFV: Efavirenz. Results are expressed as mean ± SEM. n=4

### Table 6. Effects of treatment with isoniazid and efavirenz on serum total bilirubin (mg/dl) of male albino rats

| Dose     | WK2 | WK4 | WK6 | WK8 |
|----------|-----|-----|-----|-----|
| Control  | 0.83±0.09 | 0.85±0.05 | 0.80±0.02 | 0.85±0.01 |
| INH 15mg/kg | 0.84±0.07 | 0.86±0.02 | 0.82±0.01 | 0.84±0.08 |
| EFV 10mg/kg | 0.82±0.08 | 0.84±0.01 | 0.81±0.05 | 0.80±0.04 |
| INH/EFV | 0.85±0.06 | 0.86±0.07 | 0.84±0.04 | 0.85±0.01 |

INH: Isoniazid. EFV: Efavirenz. Results are expressed as mean ± SEM. n=4

Effects on Liver Histology

Histological examination of liver of animals treated with individual doses INH and EFV and a combination of INH- EFV showed vascular congestion, inflammation of the parenchyma and degeneration of hepatocytes [Figure 2- 4].
Figure 1: Photomicrograph of H &E stained section of the liver of control rat treated with water. (x400)

Figure 2: Photomicrograph of H &E stained section of the liver of rats treated with 15mg/kg of isoniazid for 8 weeks showing vascular congestion and inflammation of the parenchyma. (x400)

Figure 3: Photomicrograph of H &E stained section of the liver of rats treated with 10mg/kg of efavirenz for 8 weeks showing vascular congestion, degeneration of hepatocytes. (x400)

Figure 4: Photomicrograph of H &E stained section of the liver of rats treated with combined doses of 15mg/kg of isoniazid and 10mg/kg of efavirenz for 8 weeks showing vascular congestion, inflammation of the parenchyma and degeneration of hepatocytes. (x400)

4. Discussion

This study, comparatively evaluated the toxicological effects of treatment with efavirenz, isoniazid and efavirenz-isoniazid (EFV-INH) combination on absolute liver weight, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), conjugated and total bilirubin levels in male albino rats. Effects on the histology of the liver were also evaluated. Toxicological and risk assessment of drugs, chemicals and other biologicals involve organ weight evaluation [29]. In this study, treatment of animals with efavirenz, isoniazid and efavirenz-isoniazid combination had no effects on absolute liver weight. Also, treatment with a combination of efavirenz-isoniazid didn’t produce synergistic toxicological effects on serum ALT, ALP, AST, conjugated and total bilirubin levels when compared to effects produced by individual doses of EFV and INH. Histopathological damage characterized by vascular congestion, inflammation of the parenchyma and hepatocytes degeneration were observed in the liver of animals treated with efavirenz, isoniazid and a combination of efavirenz-isoniazid. Observations in this study show that co-therapy with INH–EFV in HIV/TB co-infection may not be associated with synergistic hepatotoxicity. This present study, observed time-dependent increases in serum ALT, AST, ALP levels with no effects on conjugated and total bilirubin levels in EFV treated animals. Observed increases in serum ALT, AST and ALP levels in EFV treated animals suggest signs of liver damage which is consistent with the work of Kayode and others [30-33].
In addition to observations in this study, elevations in transaminases due to therapy with EFV have been also reported in humans. One of these reports is the GS 903 study which evaluated 602 naïve patients, who were randomized to receive efavirenz containing regimens for 144 weeks and reported grade 3 to 4 increase in ALT and/or AST levels [34]. Bruck et al., also evaluated 296 patients in total, of which 151 received EFV and 145 received NVP, severe hepatotoxicity of grade 3 to 4 elevation in ALT and/or AST was observed in EFV treated patients [35]. This current study also noticed time-dependent increases in AST, ALT and ALP levels without any effects on conjugated and total bilirubin in animals treated with INH. These increases in serum levels of ALT, AST and ALP suggest signs of liver damage which is in agreement with the work of Issabeagloo and Taghizadieh, who reported significant increase in liver enzymes in rats treated with INH containing anti-TB regimens for 28 days [36]. Finding in this study is also consistent with the work of Ergul and colleagues who reported increases in liver enzymes in rats treated orally with 50mg/kg of INH for 21 days [37]. Swamy and others also reported increases in liver enzymes in rats treated orally with 54mg/kg of INH for 30 days which validates our current finding [38, 39].

Furthermore, observed vascular congestion and degeneration of hepatocytes in EFV treated animals is a sign of hepatotoxicity and is consistent with some reported observations [40]. Also, some studies reported EFV associated liver damage characterized by portal and lobular inflammation particularly in zone 2 or 3 with cholestasis in zone 1 [41]. Observed vascular congestion and inflammation of the parenchyma in the liver of IHN treated animal is a sign of hepatotoxicity and is in agreement with reports from previous studies [42, 43]. Observed increases in serum ALT, AST and ALP levels in this study suggest signs of hepatic damage by these agents which might have led to increase in the permeability of cell membrane or liver systol resulting in the release of these enzymes in to the blood stream [44]. Observed elevation in liver enzymes in INH treated animals may be due to the toxic effects of INH and its metabolites [45, 46]. The metabolism of INH through acetylation and hydrolysis can lead to the production of isonicotinic acid and monoacetylhydrazine (MAH). Monoacetylhydrazine as reported can be converted to hepatotoxic species through interaction with hepatic cytochrome P450 which may be detrimental to the liver. Studies have shown that metabolic oxidation of acetylhydrazine and hydrazine can lead to the production of reactive acetylyating species which can bind to hepatic microsomal proteins and induced hepatic damage [47, 48]. Furthermore, available literature implicated INH in the induction of hepatic oxidative stress characterized by increased malondialdehyde level and decreased antioxidants [49, 50]. The involvement of oxidative stress in INH induced hepatotoxicity is supported by the fact that studies have reported the beneficial effects of treatment with antioxidants in INH induced hepatotoxicity [51-53].

Efavirenz associated hepatotoxicity has been attributed to the actions of EFV and its synthetic 8-hydroxy efavirenz (8-OHEFV) metabolite. EFV and its metabolite have been implicated in hepatic cell death, via oxidative stress and the activation of caspase-3 [54]. The involvement of hepatic oxidative stress as one of the mechanisms of EFV induced hepatotoxicity is also supported by the work of Alegre and others. They reported EFV up-regulation of hepatic endoplasmic reticulum (ER), oxidative stress markers and induction of morphological changes in ER [55]. Also, Adjene and colleagues have implicated EFV in oxidative stress induction through increased malondialdehyde level and down- regulation of antioxidants [56]. In this present study, elevated levels of transaminases can be correlated with histopathological damage observed in the liver of animals treated with these agents.

5. Conclusion

Observations in this study show that treatment with a combination of isoniazid–efavirenz did not produce synergistic toxicological effects on absolute liver weight, liver function parameters and liver histology. This study, therefore conclude that co-therapy with isoniazid and efavirenz in HIV and TB co-infection may not be associated with synergistic hepatotoxicity at the dose level used in this study.

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REFERENCES

[1] AL. Van Sighem, MA. van de Wiel, AC. Ghani, M. Jambroes, P. Reiss, IC. Gysens, K. Brinkman et al., ATHENA Cohort Study Group. Mortality and progression to AIDS after starting highly active antiretroviral therapy. AIDS, Vol. 17: 2227-2236, 2003

[2] N. Abrescia, M. D’Abbraccio, M. Figoni, A. Busto, E. Butrico, et al., Fulminant hepatic failure after the start of an efavirenz-based HAART regimen in a treatment-naïve female AIDS patient without hepatitis virus co-infection. Journal of Antimicrobial Chemotherapy. Vol. 50 No. 5, 763-765, 2002

[3] MS. Sulikowsky, DL. Thomas, SH. Mehta, RE. Chaisson, RD. Moore. Hepatotoxicity associated with nevirapine or efavirenz-containing antiretroviral therapy: Role of hepatitis C and B infections. Hepatology Vol. 35: 182-189, 2002

[4] FWN. Wit, GJ. Weaverling, J. Weel, S. Jurriaans, JMA. Lange. Incidence and risk factors for severe hepatotoxicity associated with antiretroviral combination therapy. Journal of Infect Disease, Vol. 186: 23-31, 2002

[5] G. Moyle. Efavirenz: shifting the HAART paradigm in adult
Hepatotoxic Assessment of Co-treatment with Isoniazid and Efavirenz in Albino Rats

L. Martin-Carbanero, M. Nunez, P. Rios, M. Perez-Olmeda, J. Gonzalez-Lahoz, V. Soriano. Liver injury after beginning antiretroviral therapy in HIV/hepatitis C virus-co-infected patients is not related to immune reconstitution. AIDS, 16: 1423-1425, 2002

MS. Sulikowsky, DL. Thomas, RE. Chaisson, RD. Moore. Hepatotoxicity associated with antiretroviral therapy in adults infected with Human Immunodeficiency Virus and the role of hepatitis C or B virus infection. J Am Med Ass Vol 283: 74-80, 2000

F. van Leth P. Phanuphak, K. Ruxrungthai et al. Comparison of first-line antiretroviral therapy with regimens including nevirapine, efavirenz, or both drugs, plus stavudine and lamivudine: a randomized,open-label trial, the 2NN study. Lancet Vol. 363: 1253–63, 2004

I. Sanne, H. Mommeja-Marin, J. Hinkle et al. Severe hepatotoxicity associated with nevirapine use in HIV-infected subjects. J Inf Dis Vol.191: 825–9, 2005

DP. Taneja, and D Kaur. Study on hepatotoxicity and other side-effects of antituberculosis drugs. J Indian Med Assoc, Vol.88 : 278-80, 1990

T. Schaberg, K. Rehan, H. Lode. Risk factors for side-effects ofisoniazid rifampin and pyrazinamide in patients hospitalized for pulmonary tuberculosis. European Respiratory Journal Vol. 9: 2026-30, 1999

K. Nanashima, T. Mawatari, N. Tahara, N. Higuchi, A. Nakaura A, et al. Genetic variants in antioxidant pathway: risk factors for hepatotoxicity in tuberculosis patients. Tuberculosis, Vol. 92: 253-259, 2012

S. Santhosh, TK. Sini, R. Anandan, PT. Mathee. Hepatoprotective activity of chitosan against isoniazid and rifampicin-induced toxicity in experimental rats. European. Journal of Pharmacology., 572:69-73, 2007

SS. Wu, CS. Chao, JH. Vargas, HL. Sharp, MG. Martin, SV. McDiamid, et al. Isoniazid-related hepatic failure in children: a survey of liver transplantation centers. Transplantation. Vol. 84:173-9, 2007

JR. Itchel, HJ. Zimmerman, KG. Ishak, UP. Thorgerisson JA, Timbrell Isoniazid liver injury clinical spectrum, pathology, possible pathogenesis. Ann Intern Med Vol 84; 181-92, 1976

A. Pandit, T. Sachdeva and P. Bafna. Drug-Induced Hepatotoxicity: A Review. Journal of Applied Pharmaceutical Science Vol.5: 233-243, 2012

Global report: UNAIDS report on the global AIDS epidemic 2013 [http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013.UNAIDS_Global_Report_2013_en.pdf]

Time to act. Save a million lives by 2015. Prevent and treat tuberculosis among people living with HIV [http://www.stoptb.org/assets/documents/resources/publications/acsm/TB_HIV_Brochure_Singles.pdf]

B. J. Larru,Eby, ED. Lowenthal, Antiretroviral treatment in HIV-1 infected pediatric patients: focus on efavirenz. Pediatric Health, Medicine and Therapeutics Vol. 5 29–42, 2004

FY. Khan, and F Rasoul . Rifampicin-isoniazid induced fatal fulminant hepatitis during treatment of latent tuberculosis: A case report and literature review. Indian J Crit Care Med Vol.14:97-100, 2010

J. Yue , RX. Peng, J. Yang, R. Kong, J. Liu. CYP2E1 mediated isoniazid-induced hepatotoxicity in rats. Acta Pharmacol Sin Vol. 25:699-704, 2004

International, national and institutional guidelines for care and use of laboratory animals in biomedical research as promulgated by the Canadian Council of Animal Care (2009)

Treatment of Tuberculosis and Tuberculosis Infection in Adults and Children, Am J Respir Crit Care Med Vol 149, pp 1359-1374

G. K, Bertram Basic and Clinical pharmacology, 9th edition pp.818

Goodman and Gilman’s. The pharmacological basis of therapeutics 11th Edition, PP. 120-1207.

S. Reitman, and S. A. Frankel, colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology, Vol. 28, 56-63, 1975

E. J. King and P. R. N Kind, Estimation of plasma phosphatase by determination of hydrolyzed phenol with amino-antipyrene. J. Chem. Path. Vol. 7: 322-326., 1957

I. Ogubuehi, E. Adikwu and Oputiri. Effect of Acalypha wilkesiana MullArg Leaf Extract on the Oxidative Indices, Liver Enzymes and Liver Integrity of Rats Infected with Plasmodium berghei . British Journal of Pharmacology and Toxicology 5(2): 68-74, 2014

B. Michael, B. Yano, RS. Sellers, R. Perry, D. Morton, N. Roome et al. Evaluation of Organ Weights for Rodent and Non-Rodent Toxicity Studies: A Review of Regulatory Guidelines and a Survey of Current Practices. Toxicol. Pathol. Vol. 35, No. 5:742-750,2007

G. M. Liss, R.A. Greenberg and C.H. Tamburro, Use of serum bile acids in the identification of vinyl chloride hepatotoxicity. Am. J. Med., 78: 68-76, 1985

C. Robert and TR. Hustead, Causes and Evaluation of Mildly Elevated Liver Transaminase Level Am Fam Physician., Vol. 84, No. 9, 1003-1008, 2011

R. Manfredi, L. Calza, F. Chioldo. Efavirenz versus nevirapine in current clinical practice: A prospective, open-label observational study. J Acquir Immune Defic Syndr Vol 35: 492–502, 2004

A. A. Kayode, OT. Kayode, OA. Aroyeu, and MC. Stephen, Hematologic and Hepatic Enzyme Alterations Assinated with Acute Administration of Antiretroviral Drugs. Journal of Pharmacology and Toxicology, Vol. 6: 293-302., 2011

JE. Gallant, S. Staszewski, AL. Pozniak, et al. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naïve patients: a 3-year randomized trial. JAMA, Vol 292:191-201, 2004

S. Brück, . And S. Witte, J. Brust D. Schuster, F. Mosthaf, M. Procaccianti, JA. Rump, hepatotoxicity in patients prescribed efavirenz or nevirapine, Eur J Med Res (2008) 13: 343-348
A. Turkova, C. Ball, S. Gilmour-White, M. Rela and G.
Clinical Medicine Insights: Pediatrics
Failure in Children Treated for Latent Tuberculosis Infection
Boston, MA, USA November 7 - 11 2014
American Association for the Study of Liver Diseases,
liver injury: a cohort analysis 65th Annual Meeting of the
CW. Spearman  Characteristics of Efavirenz drug induced
2009
Antimicrob Chem Advance Access published January 22,
antiretroviral treatment after liver transplantation Jour of
therapy and effective use of raltegravir in combination
associated with efavirenz-based highly active antiretroviral
Mieli-Vergani A paediatric case of acute liver failure
2014
C. Singh, J. Laxmikant TD. Bhatt, MS. Gill, S. Suresh S,
Hepatoprotective agent tethered isoniazid for the treatmentof
drug-induced hepatotoxicity: Synthesis, biochemical and
histopathological evaluation Toxicology Reports Vol.1,885–
93, 2014
S. Sarkar, P. Yadav and D. Bhatnagar,. Lipid peroxidative
damage on cadmium exposure and alterations in
antioxidantsystem in rat erythrocytes: A study with relation
to time. Biometals, Vol. 11: 153-157, 1998
EL. Saad, SM. El-Gowilly, MO. Sherhaa, AE. Bistawros,
Role of oxidative stress and nitric oxide in the protective
effects of α-lipoic acid and aminoguanidine against
isoniazid-rifampicin- induced hepatotoxicity in rats.Food
Chem. Toxicol., Vol. 48: 1869-1875, 2010
X. Chen, J. Xu C., Zhang, T. Yu, H. Wang H, et al. The
protective effects of ursodeoxycholic acid on isoniazid plus
rifampicin induced liver injury in mice.European. Journal of
Pharmacology. Vol. 659: 53-60, 2011
BHI. Thomas , LT. Wang, ZW. Zent, G. S Raj, Isoniazid
metabolism in the rabbit and effect of rifampicin pretreatment
Res Commun, Chem Pathol, Pharmacol Vol. 1, No.
33;235-47, 1987.
A. Noda, h, Hsuky, h. Noda, Y. Yamamoto, T. Kurozumi is
isoniazid-hepatotoxicity induced by metabolites hydralazine J.
UOEH, Vol.5; 183-90, 1983.
CP. Sodhi , SV. Rana , SK. Mehta, K. Vaiphei, S. Attr, S.
Thakur, S. Mehta. Study of oxidative stress in
isoniazid-induced hepatic injury in young rats with and
without protein-energy malnutrition. J Biochem Toxicol Vol.
11:139–146, 1996
CP. Sodhi , SV. Rana , SK. Mehta, K. Vaiphei, S. Attr, S.
Mehta Study of oxidative-stress in isoniazid-rifampicin
induced hepatic injury in young rats. Drug Chem. Toxicol.
(1997); 20: 255–69.
S. Attr S.V.Rana, K. Vaiphei, CP. Sodhi , R. Katyal, R.C.
Goel, CK. Nain, Isoniazid – and rifampicin–induced
oxidative injury hepatic injury – protection by N–
acyethylcysteine Human and Experimental Toxicology, Vol. 19 No. 9 517-522,
2000
V. Tayal, BS. Kalra, S. Agarwal, N. Khurana, U. Gupta,
hepatoprotective effect of tocopherol against isoniazid and
rifampicin induced hepatotoxicity in rabbits. Indian Journal.
of Experimental Biology , Vol45; 1031-1036, 2007
S. Baniasadi , P. Eftekhari , P. Tabarsi , F. Fahimi , M R.
Raoufy et al . Protective effect of N -acetylcysteine on
isoniazid-rifampicin-induced acute liver failure in young rats.
Human and Experimental Toxicology, Vol. 5;9-15, 2011
Y. Issabeagloo, and M Taghizadieh, Hepatomodulatory
Action of Camellia sinensis Aqueous Extract against
Isoniazid-Rifampicin Combination Induced Oxidative Stress
in Rats Advances in Bioresearch, Vol 3:18-27,2012
E Ergul, T. Erkan , H. Uzun, H. Genc, T. Altug andE. Erginoz,
Effect of vitamin C on oxidative liver injury due to
isoniazid in rats Pediatrics International, Vol5, 2, 69–74, 2010
AV. Swamy, RV. Kulkarni, AM. Thippeswamy, BC. Koti,
and A. Gore, evaluation of hepatotoxicity activity of cissus
quadrangularis stem extract against isoniazid-induced liver
damage in rats. Indian Journal of Pharmacology, Vol. 42; 397-400, 2010
D. Desrochers, RP. González-Peralta, DT. McLenathan, MJ.
Wilsey, and A. Haafiz Isoniazid-Induced Severe
Hepatotoxicity: An Infrequent but Preventable Cause of Liver
Failure in Children Treated for Latent Tuberculosis Infection
Clinical Medicine Insights: Pediatrics,Vol.5:9-15, 2011
A. Turkova, C. Ball, S. Gilmour-White, M. Rela and G.
Mieli-Vergani A paediatric case of acute liver failure
associated with efavirenz-based highly active antiretroviral
therapy and effective use of raltegravin in combination
antiretroviral treatment after liver transplantation Jour of
Antimicrob Chem Advance Access published January 22,
2009
MW. Sonderup, H. Wainwright, D. Maughan, M. Setshedi,
CW. Spearman Characteristics of Efavirenz drug induced
liver injury: a cohort analysis 65th Annual Meeting of the
American Association for the Study of Liver Diseases,
Boston, MA, USA November 7 - 11 2014
GI, Qader , R. Aziz, ZA. Ahmed , Z, Abdullah, and SA.
Hussain, “Protective Effects of Quercetin against Isoniazid
and Rifampicin Induced Hepatotoxicity in Rats.” American
Journal of Pharmacological Sciences, Vol. 2, no. 3 : 56-60,
2014
C. Singh, J. Laxmikant TD. Bhatt, MS. Gill, S. Suresh S,
Hepatoprotective agent tethered isoniazid for the treatmentof
drug-induced hepatotoxicity: Synthesis, biochemical and
histopathological evaluation Toxicology Reports Vol.1,885–
93, 2014
S. Sarkar, P. Yadav and D. Bhatnagar,. Lipid peroxidative
damage on cadmium exposure and alterations in
antioxidantsystem in rat erythrocytes: A study with relation
to time. Biometals, Vol. 11: 153-157, 1998
EL. Saad, SM. El-Gowilly, MO. Sherhaa, AE. Bistawros,
Role of oxidative stress and nitric oxide in the protective
effects of α-lipoic acid and aminoguanidine against
isoniazid-rifampicin- induced hepatotoxicity in rats.Food
Chem. Toxicol., Vol. 48: 1869-1875, 2010
X. Chen, J. Xu C., Zhang, T. Yu, H. Wang H, et al. The
protective effects of ursodeoxycholic acid on isoniazid plus
rifampicin induced liver injury in mice.European. Journal of
Pharmacology. Vol. 659: 53-60, 2011
BHI. Thomas , LT. Wang, ZW. Zent, G. S Raj, Isoniazid
metabolism in the rabbit and effect of rifampicin pretreatment
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33;235-47, 1987.
A. Noda, h, Hsuky, h. Noda, Y. Yamamoto, T. Kurozumi is
isoniazid-hepatotoxicity induced by metabolites hydralazine J.
UOEH, Vol.5; 183-90, 1983.
CP. Sodhi , SV. Rana , SK. Mehta, K. Vaiphei, S. Attr, S.
Thakur, S. Mehta. Study of oxidative stress in
isoniazid-induced hepatic injury in young rats with and
without protein-energy malnutrition. J Biochem Toxicol Vol.
11:139–146, 1996
CP. Sodhi , SV. Rana , SK. Mehta, K. Vaiphei, S. Attr, S.
Mehta Study of oxidative-stress in isoniazid-rifampicin
induced hepatic injury in young rats. Drug Chem. Toxicol.
(1997); 20: 255–69.
S. Attr S.V.Rana, K. Vaiphei, CP. Sodhi , R. Katyal, R.C.
Goel, CK. Nain, Isoniazid – and rifampicin–induced
oxidative injury hepatic injury – protection by N–
acyethylcysteine Human and Experimental Toxicology, Vol. 19 No. 9 517-522,
2000
V. Tayal, BS. Kalra, S. Agarwal, N. Khurana, U. Gupta,
hepatoprotective effect of tocopherol against isoniazid and
rifampicin induced hepatotoxicity in rabbits. Indian Journal.
of Experimental Biology , Vol45; 1031-1036, 2007
S. Baniasadi , P. Eftekhari , P. Tabarsi , F. Fahimi , M R.
Raoufy et al . Protective effect of N -acetylcysteine on
isoniazid-rifampicin-induced acute liver failure in young rats.
Human and Experimental Toxicology, Vol. 5;9-15, 2011
N. N. Bumpus Efavirenz and 8-hydroxyefavirenz induce cell
death via a JNK- and BimEL-dependent mechanism in
primary human hepatocytes.Toxicology and Applied
Pharmacology, Vol. 257, No., 2, 227–234, 2011
F. Alegre, M. Polo, L. J. Go’mez-Sucerquia,  H. A. Funes
H. B. Garcia, et al., efavirenz induces endoplasmatic stress in
human hepatic cells by a mechanism different than that
elicited by the pharmacological inducer thapsigargin, Journal
of Hepatology Vol. 58, S63–S227, 2013
JO. Adjene , JA. Aivbunudiogba, PS, Igbibgi, Oxidative stress
induced by chronic administration of Efavirenz on the
intracranial visual relay centers of adult Wistar rats
Biology and Medicine, Vol. 3 (5): 16-24, 2011