International Journal of Basic Science and Technology
January, Volume 7, Number 1, Pages 10 - 14

ISSN 2488-8648
http://www.ijbst.fuotuoke.edu.ng/10

Subacute Effects of Isoniazid (Inh) on The Kidney and Liver of Albino Mice

[1]Omorodion, N.T., [1]Henry, A. and [2]Achukwu, P.U

[1]Department of Medical Laboratory Sciences, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin city, Edo state, Nigeria[1]
[2]Department of Medical Laboratory Sciences, Faculty of Health Science and Technology, College of Medicine, Enugu campus, University of Nigeria, Enugu state, Nigeria

Article Information

Article # 02001
Received: 12th June, 2020
Revision: 4th Dec., 2020.
Acceptance: 8th Jan., 2021
Published: 20th Jan., 2021

Key Words
Subacute, Isoniazid, Kidney, Liver, Albino Mice.

Abstract

The subacute effects of Isoniazid on the kidney and liver were determined using twenty-five Albino white mice. The mice were sex- matched and grouped into 5 cages of five mice each. Lesser dose of the known LD_{50} of isoniazid graded into 0mg/kg, 10mg/kg, 20mg/kg, 40mg/kg and 60mg/kg were administered through their distilled water. They were exposed to water and Guinea feed© libitum for 49 days. Each mouse was weighed on day 1 and observed daily. On day 49, the final weights of the mice were measured and two mice were selected at random from each cage and sacrificed. Their kidney and lever were harvested, examined grossly and processed using standard histological techniques for light microscopy. The results revealed no significant weight change. Grossly, the organs showed brown induration. Microscopically, significant structural disorders of both organs were observed with the exception of the group administered with 10mg/kg. Speculatively these effects can be extrapolated to man, thus similar damage to human kidney and liver may be possible. Therefore, their administration should be carefully monitored.

*__Corresponding Author__: **Omorodion, N.T.**; terry.omorodion@uniben.edu

Introduction

Isoniazid is a first line antituberculosis medication used in the prevention and treatment of tuberculosis (TB). It is often prescribed under the name INH. The chemical name is isonicotinyl hydrazine or isonicotinic acid (Bass *et al.*, 1995). It is available in tablet, syrup and vial forms given via intramuscular injection, available worldwide and inexpensive to produce.

Since isoniazid is increasingly being used to control the spread of tuberculosis, physicians must be aware of its potentially fatal effects (Byrd *et al.*, 1979). Most physicians are aware of the use of liver function tests to detect hepatotoxicity in patients with tuberculosis who are being treated with isoniazid (Brennan and Nikaido, 1995; Gumbo, 2011). However, physicians may not be aware that the acute ingestion of as little as 1.5g of this drug can be toxic. The large quantities of the drug (80-150mg per kg or more) are taken intentionally or accidentally, recurrent seizures, profound metabolic acidosis, coma and even death can occur (Byrd *et al.*, 1979).

For most patients, the proffered regimen for treating TB disease consists of an initial two months phase of four drugs: Isoniazid and rifampin (Houston and Fanning, 1994). Streptomycin may be substituted for ethambutol but be given by injection. In areas where the rate of isoniazid resistance is documented to be less than 4% and the patient has had no previous treatment with TB drug, is not from a country with a high prevalence of drug resistance and has no known exposure to a patient with drug resistance disease, three drugs: Isoniazid, rifampin and pyrazinamide may be adequate for the initial regimen (Iseman, 1993; Mitchison, 2000)

The term tuberculosis is generally restricted to disease caused by Mycobacterium tuberculosis and Mycobacterium bovis. Other mycobacterial infections (so called atypical or non-tuberculosis mycobacteria) may produce clinicopathologically similar disease (Houston and Fanning, 1994).

The tubercle bacillus is a long, thin rod that grows in sinuous masses or strands called cord. Unlike many bacteria, it produces no exotoxins or enzymes that contributes to infectiousness. Most strains contain complex waxes and a cord factor that contribute to virulence by preventing the mycobacteria from being destroyed by the lysosomes or macrophages. Their survival contributes to further invasion and persistence as intracellular parasites (Christie *et al.*, 2001).

As the incidence of tuberculosis increases, physicians will continue to prescribe more isoniazid. Isoniazid is an over-the-counter drug, therefore is commonly procured and taken at will hence it fetters quite easily into the food chain of human as in our environment.

Isoniazid is a hydrazide derivative which is the mainstay of primary treatment of pulmonary' and extra pulmonary tuberculosis (Dutt and Stead, 1996). TB must be treated for a long time at least 6 months for most patients compared with many other infectious diseases. If treatment is not continued for a sufficient length of time, some tubercle bacilli may survive and revive thus the patient may become ill and infectious again (Combs, 1990). Isoniazid is therefore a long-term consumed drug having its side effect, his brings about the reason for this study, which therefore is to: Investigate the effect of Isoniazid on the physical parameters using experimental animals model of Albino white mice, Characterize grossly and microscopically the effect of Isoniazid on the liver and kidney of the animal model within the exposed time.

Materials and Methods

A total of twenty-five albino mice of both sexes aged 2-3months old were used. They were obtained in the animal house of University of Nigeria Teaching Hospital, Enugu.

Isoniazid of 300mg manufactured by Vardman Export India was used.

Ethical consideration

The study ethical approval was granted by College of Medicine Ethical Committee (COMREC), University of Nigeria. The study was also conducted in compliance with policies outlined in the Guide for the Care and Use of Laboratory Animal (NRC, 2012).

Design and Conduct of Experiment

This study is designed with five groups of sex matched Albino mice. They were fed for two weeks for the purpose of acclimatization with pelleted Guinea Feed. Four groups labeled A-D were given varying doses of Isoniazid less than the LD_{50} as reported by WHO (2000) through distilled water ad libitum in the proportion of 60mg/kg, 40mg/kg, 20mg/kg and 10mg/kg respectively for 49 days. The fifth group E not subjected to any dose administration served as control. The water was pyrogen free. The drug was measured using Mettler's sensitive weighing balance. They were kept in stainless steel wire mesh cages, which separated the animals from their faeces to prevent coprophagy. Their weight was taken before administration to the nearest whole number and observed for physical changes daily during administration. The weight and doses of this work is represented below.

Table 1: Dose and Weight of the Animals before Administration

Cage	Sex	No of Mice	Dose (mg/kg)	Initial Av weight (g)	No of days of admin.
A	Male	5	60	46	49
B	Female	5	40	49	49
C	Female	5	20	51	49
D	Female	5	10	49	49
E	Male	5	-	51	-

On day 49, two mice were randomly selected from each group and painlessly sacrificed with the aid of chloroform. The liver and kidney of the selected mice were harvested using dissecting set and observed gross anatomically and processed histologically using standard histological procedure.

Processing of Sample for Histology

The excised organs were cleared of blood vessels and fixed in 10% buffered formalin for 48 hours. About 5mm thick of tissue was cut from the gross specimen and processed for light microscopy

The tissues were sectioned at 5pm thick in duplicate using Heitz Rotary microtome. Tissue slide sections were stained using Erhlich's haematoxylin technique of 1935 as modified by baker and Silverton 1998.

Microscopy and Photomicrography

The sections were examined using Binocular microscope with inbuilt light source. The section's micrographs were photographed using Black and White 35mm film with an Olympus photomicroscope.

Results

Physical Parameters

The initial and final mean weights of the animals before and after drug administration were compared as

represented below. The P-value was calculated using student's t-table.

Table 2; Comparisons of Weights of Animals Before and After Experiment for 49 Days

Age	Dosage admin (mg/kg)	Initial weight (g)	Final weight(g)	P
A	60	46.011±2	44.411±1	P>0.05
B	40	48.814±5	47.014±4	P>0.05
C	20	51.213.6	50.813±1	P>0.05
D	10	49.414±4	49.014±8	P>0.05
IT	0	50.6+3±0	52.012±6	P>0.05

From the table above, the result showed no significant change in the weight of the test animals in the pre- and post-treatment periods. Other physical parameters observed tor were skin reactions, fatigue and vomiting and the animals showed no such adverse effects.

Cross Macroscopy of the Organs

The fiver and kidney of the test animals after having been harvested when compared with control showed brown in-duration of the organs.

Morphological Results

Histological features of the liver and kidney were examined using Binocular Olympus® microscope, which were photographed. The result is as represented in the table below.

Table 3: Histological Feature Observed in Kidney

Dosage admin Istration (mg/kg)	↑ UP	C	ET	IC
60	+	+	+	+
40	+	+	+	+
20	±	±	±	±
10	-	-	-	±
0	-	-	-	-

Keys ; ↑UP = Increased urinary pole; C = Constricted capillary tuft; ET = Elongated tubule;IC = Inflammatory cells

The above table indicates that the features observed were the presence of increased urinary pole, constricted capillary tuft, elongated tubule and inflammatory cells in the groups administered with 60mg/kg and 40mg/kg of Isoniazid. Presence of inflammatory cells was also observed in the group administered with 20mg/kg while other features were not significant.

These features were absent in the group administered with 10mg/kg of Isoaiazid and the control, which was not administered with any dose of Isoniazid. However, the presence of inflammatory ceil which was not significant, was observed in the group administered with 10mg/kg of isoniazid.

The table above shows the presence of frank cells, vacuolation and haematoma which was observed in all the groups including the control. The presence of inflammatory cells and necrosis was observed in groups administered with 60mg/kg and 40mg.kg but was not significant in the group administered with 20mg/kg and was not present in the group administered with 10mg/kg and the control.

Discussion

The effects of prolong use of 1NH, as a frontline drug for the treatment of TB has not been commonly observed. This is because of the joy of recovery from the draconian claws of TB that seems to overwhelm the subliminal tissue damage associated with this drug (Mdluli, 1998). For instance, INH has been acclaimed to be one of the safest antibiotics of choice to drastically reduce the dangers of TB among sufferers worldwide (WHO, 2000).

INH continues to be a highly effective drug in die chemoprophylaxis and treatment of TB, however its use is associated with hepatotoxicity, predominantly hepatic necrosis as has been shown by this investigation. The INH metabolites acetylhydrazide

and hydrazine have each been implicated as the causative hepatotoxin in INH-induced hepatotoxicity as was recorded by Timbrell *et al.,* (2004). In his work he used a model of INH-induced hepatotoxicity in rabbits in which INH-induced hepatotoxicity manifested as hepatic necrosis, hepatic steatosis {heapatic fat accumulation) and hypertriglyceridaemia (elevated rlasrr.a triglycerides)'. He compared the severity of these measures of toxicity with plasma levels of INH, acetylhydrazine and hydrazine.

His result showed that INH-induced hepatotoxicity in rabbits that hydrazine and not INH or acetylhydrazine is most likely involved in the pathogenic mechanism of hepatic necrosis. This result however was different from that of Nebert (1977) in which he determined the relationship between the hepatotoxicity and metabolism of Isoniazid and its metabolites, acetylisoniazid and acetylhydrazine.

Toxic doses of acetylisoniazid and acetylhydrazine, radiolabeled in the acetyl group were found to bind covalently to liver protein in vivo. This binding was mediated by the microsomal enzyme system.

His metabolic studies revealed that the pretreatments increased the metabolism of the acetylhydrazine moiety of acetyl-labeled acetylisoniazid and acetylhydrazine itself by the microsomal enzyme system. His results strong suggest that acetylhydrazine is the metabolite responsible for the hepatic necrosis caused by isoniazid and that microsomal metabolism of acetylhydrazine in vivo leads to the production of a reactive acylating species capable of reacting covalently with tissue macromolecules.

Be it acetylhydrazine or hydrazine, these works have explained above implicates that Isoniazid is toxic to the liver parenchyma. No doubt in a U.S Public Health Service Surveillance study involving 13,838 persons taking INH there were 8 deaths among 174 cases of hepatitis.

The kidney however is not left out since these toxic metabolites are excreted via the kidney leading to distortion of both the physiology and anatomy of the kidney.

As has been shown by this work, these toxic metabolites bring about the infiltration of inflammatory cells and increased urinary pole leading to tissue necrosis, thus improper functioning or loss of function of the kidney.

Though much work has not been done on the effects of isoniazid on kidney, it has been reported that isoniazid-induced nephritis is up to 1.5% (Robert *et al*., 2000).

In addition, tissue damages on the kidney and liver of the mice was very minimal giving a fair intact visceral organ. This agrees with the work of CDC (1999), which stipulates a constant internal organ in relation to that of the overall weight when the visceral organs were intact.

The non-change in weight and absence of other adverse effects on the mice could be as a result of proper and normal feeding of the mice.

Until now, there has been reported increase in TB cases (Havliv and Barnes, 2000). TB must be treated for a long period at least 6 months and Isoniazid is still the mainstay treatment against TB and continues to be the first line

However, the effects of the drug on the tissue have not been fully elaborated since INH, which is metabolized in the liver via acetylation and hydrolysis produce two toxic metabolites, acetylhydrazine and hydrazine, which are excreted in the urine via the kidney (Evans, 2000; Maddrey and Boitnott 1993).

Conclusion

This work therefore serves as a baseline for further researches on effects of prolong use of INH. It also suggests that patients given Isoniazid should becarefully monitored and interviewed regularly. They should be instructed to report immediately any of predomal symptoms of hepatitis such as fatigue, weakness, malaise, anorexia, nausea or vomiting. If symptoms and signs suggestive of hepatic damage are detected, an alternative agent should be used since continued use of Isoniazid in these patients may cause a more severe form of liver damage

References

Baker, F.J. and Silverton, R.E. (1998). Staining procedures; Introduction to Medical Laboratory Technology 6lh ed. Butterworth- Heinemann. 223-224

Bannerjee, A., Derby, L.E., Jiclc, H., Dean, A.D., Dzuikas, L.J. and Vohra, J. (1994). A gene encoding a target for Isoniazid and ethionamide in Mcobacteriumtuberculosis. *Journal of Pharmacology and therapeutics*; 126:25-100

Bass, J.B., Bickel, W.K., Braga, P.C., Crain, S.M., Shen, K.F. (1995). Treament of tuberculosis and tuberculosis in infection in adults and children. *American Journal of Critical Care Medicine*, 149: 1359-1588

Bemadou, J., Nguyem, M. and Meunier, B. (2001). The Mechanism of action of Isoniazid: A chemical model of activation. *Journal Tuberculosis*; 52:221-408

Brennan, P.J. and Nikaido, H. (1995). The Envelop of mycobacteriua. Review of Biochemistry, 64: 29-100

Bryd, R.B., Evans, C.J, Ferrante, F.M,, Fields, H.L and Foley, K.M. (1979). Toxic effects of Isoniazid in tuberculosis chemoprophylaxis. *Journal of Neurochemistry*; 241: 1239-1487

Center for Disease Control and prevention (1999). Prevention and Treatment of tuberculosis among patients infected with HIV. Principles ot therapy and recommendations: Morbid Mortality Weekly report (MMWR) 47: (RR-20):

Christie, A.B., Lucas, S.B., Jopling, V.V.H. Me Dougall, A.C. and Frema, B.C. (2001). Infectious diseases: Epidemiology and clinical practice. *Journal of Pharmacology and Therapeutics*; 44: 584-671

Combs, D.L., Mather, L.E., Hollt, V., Irwin, R.S., Curley, F.J. and Benneth, F.M. (1990). Tuberculosis, Short course chemotherapy trial 21. *Annual International Medici*; 397-512

Cooper, A.M. and Flynn, J.L. (1995). The protective Immune response to M. tuberculosis. *Current Opinion on Immunology*; 7: 512-589

Dayer, P., Farrar, H.C., Wells, T.G., Kearns, G.L. and Wada, Y. (1993). Contribution of the genetic status of oxidative metabolism to variability in the plasma concentrations* of beta-adrenoceptor blocking agent. *England Journal*; 24: 797-842

Dutt, A.K., Moers, D. and Stead, W.W. (1983). Undesirable side effects of Isoniazid and Rifampin in largely twice-weekly short course chemotherapy for tuberculosis. American Review Respiratory Disease; 128: 419-700

Dutt, A.K., Moers, D. and Stead, W.W. (1996): Short course chemotherapy for Extrapulmonary tuberculosis. Annual International Medicine; 104: 7-84

Gumbo, T., Brunton, L,L., Chabner, K.A., Knollman, K.C. (2011). Chemotherapy of tuberculosis, mycobacterium avium complex disease and leprosy In Goodman & Gilman's the pharmacological basis of therapeutics. 12th ed. New York: McGraw-Hill, 1549-1570

Evans, D.A.P., Manley, K.A. and McKussick, V.A. (2000). Genetic control of Isoniazid metabolism in man. *British Medical Journal*; 2: 485- 802

Goble, M. and Ellard, G.A. (1998). Chemotherapy of Tuberculosis. *Journal of Medicine*; 149: 166-190

Havliv, D.V. and Barnes, P.F. (2000). Tuberculosis in patients with Human Immunodeficiency virus infection. *North England Journal of Medicine*; 340: 367-815

Horsburgh, C.R. (1991). Mycobacterium avium complex in AIDS. *North England Journal of Medicine*; 324: 1332-1423

Houston, S. and Fanning, A. (1994): Current and Potential Treatment of Tuberculosis. *Drugs*', 48: 689-800

Iseman, M.D. (1993). Treatment of Multidrug resistant tuberculosis. *England Journal of Medicine*; 329: 784-812

Maddrey, W.C. and Boitnott J.K. (1993). Isoniazid hepatitis. *Annual International Medicine*; 79: 1-180

Mdluli, K. (1998). Inhibition of a mycobacterium tuberculosis beta keto-acyl ACP synthase by Isoniazid. *American Journal*;280:1607-1789

Minchin, R.F. and Boyd, M.R. (1999). Localization of metabolic activation system of Xenobiotics. *Annual Review*. 23:217-302

Mitchison, D.A. (2000). The action of antituberculosis drugs in short course chemotherapy. *Tubercle Annual Review on Biochemistry*; 66: 219-291

Nebert, D.W. (1997). Polymorphism in drug metabolizing enzymes. What is their clinical relevance and why do they exist? *American Journal of Human Genetics*; 60:1265-2034

National Research Council (NRC). Nutrient requirements of fish and shrimp. *Aquacult Int*. 2012; 20: 601–602 . https://doi.org/10.1007/s10499-011-9480-6

Omorodion, N.T., Atoigwe-Ogeyemhe, E. B., Achukwu, P.U. Odigie, E. B. (2019). Histopathological changes associated with exposure of some viscerals and testicular tissues to bisephenol A and di(2-ethylhexyl)phthalate *Trop J Pharm Res.*18(6): 1213-1218

Robert K.M, Daryl K.G, Peter A.M and Victor W.R (2000) Conjugation reactions preparexenobiotics for excretion in Phase II of their metabolism. Helper's Biochemistry 25lh ed. McGraw Hill, NewYork. 782-784

Timbell. J.A., Mitchell J.R., Snodgrass W.R. and Nelson, S.D. (2004). Isoniazid hepatotoxicity. Toxicological Sciences; 18: 1432-1738

World Health Organization (2000). Infectious Disease. Medical Hygiene; 84: 1432-1738

Yabrough, B.E. and Wood, S.P. (1983). isoniazid overdose treated with high dose pyridoxin. Emergency Medicine, 12:303-527

Zander, U.M., Pappano, A.J., Mubagwa,. K, Ohia, S.E., Jumblatt, J.E. (1998). Absence of hepatic cytocrome P-450 causes genetically deficient debrisoquin oxidation in man. Journal of Infectious disease; 4: 902-936