Isoniazid Induced Toxicities and Idiosyncratic Responses in Male Albino Wistar Rats

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Abstract: Isoniazid (INH) is an anti-tuberculosis drug administered over a long period. Upon metabolism in the liver, INH generates nitrogen-centered radicals, reacting with cellular macromolecules, and induces toxic and transformational changes in cells and tissues. Here we examined the side effects of long-term (chronic) administration of isoniazid (2.5 and 5mg/kg) once daily for 30, 60 and 90 days consecutively: on hepatic transaminases, histological changes in hepatocytes and induction of micronuclei in the bone marrow and possible genotoxicity in *E. coli* PQ37. In addition, blood glucose was monitored during the various treatment period.

Biochemical analysis of hepatic transaminases (γ -glutamyl-, alanine amino-, aspartate aminotransferases and alkaline phosphatase) in INH treated group was significantly *($p<0.05$)* elevated as well as blood glucose level declined significantly *($p<0.05$)* for both doses at 30, 60 and 90 treatment respectively. Total protein and albumin level decreased *($p<0.05$)* at both treatment doses compared to control. Serum creatinine level significant ($p<0.05$) increased at days 30 and 60 relative to control.

Antioxidants (GST, SOD and catalase) activity examined indicated a decrease in catalase levels which was significant ($p<0.05$) and an insignificant decrease ($p>0.05$) in GST in both treatment groups at day 60. There was also a significant increase ($p<0.05$) in the activity of superoxide dismutase activity. Micronucleus analysis further revealed an induction of micronucleated polychromatic erythrocytes (mPCEs), which was significant ($p<0.05$) for both treatment doses at days 30, 60 and 90 respectively. In addition, INH genotoxicity assessed by UMU chromotest indicated that the 5mg/kg dosage has an induction ratio above the genotoxicity threshold of 1.5 suggesting genotoxicity in *E.coli* PQ37.

Taken together, INH treatment at both doses (2.5 and 5mg/kg body weight) was hepatotoxic and induced nephrotoxic damages, in addition to mutagenic effect which is more pronounced at 2.5mg/kg dose, thereby suggesting dose-dependent cellular and genetic toxicity.

Keywords: Isoniazid, Tuberculosis, *Mycobacterium tuberculosis,* hepatotoxicity, genotoxicity, carcinogenesis.

INTRODUCTION

Tuberculosis (TB) is a disease that has plagued humanity from antiquity. Until date, it has been identified as a disease like no other, which has most afflicted humanity considering its fatal morbidity and mortality rate [1]. TB is caused by *Mycobacterium tuberculosis*, the most notable member of a large class of *actinomycetes* of the genus *Mycobacterium* that evolved to be resident in mammalian host. The similarity among this class of organisms is about 99.9% [2]. However they differ from each other considering the host type, mode of infection and disease causation among many others [3].

Precise estimation of latent tuberculosis infection globally has been difficult to ascertain, however some modeling studies suggest that over 2 billion people are infected global with latent tuberculosis [4]. Several factors determine the tendency of progression of latent tuberculosis infection to active tuberculosis disease and they range from bacterial, to the host, and other environmental influences [5]. Also known to increase progression risk include HIV infection, which suppresses cellular immunity [6], organ [7] or hematologic transplantation [8].

According to Cruz-Knight and Blake-Gumbs, the first-line drugs employed in the treatment and management of tuberculosis includes isoniazid, pyrazinamide, rifampicin and either ethambutol or streptomycin. A second-line of drugs which are subsidiary or follow-up drugs include amikacin, kanamycin or capreomycin [9]. INH is preferably in the management of TB in Nigeria, administered daily, for 6-12 months, and has been notably efficacious with a range of about 60-90% [10]. However, the lengthy administration of INH raises toxicological questions, which this present study attempts to address: the genotoxic effects of long-term isoniazid administration and a likely role in hepatocarcinogenesis among other unintended effect.

MATERIALS AND METHODS

Chemicals

Alanine amino transferase (ALT), aspartate amino transferase (AST) and gamma glutamyl transferase (γ-

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GT) were purchased from CYPRESS Laboratory, (Leuven, Belgium) and other reagents and chemicals used in the course of this study are of analytical grade and were obtained from Sigma Chemical Co. (St. Louis, MO., USA).

Test Material Isoniazid (INH)

INH tablets (300mg) (Tradename: Conazide) were obtained from Diadem Pharmaceuticals, Ibadan, Oyo State, Nigeria. The tablet (300mg) was dissolved in 120ml and 240ml distilled water to obtain 5mg and 2.5mg/kg solution respectively. INH solution was preserved at 4-8°C until needed for dosing.

Experimental Animals

Sixty male albino Wistar rats showing no visible signs of diseases or injuries and weighing between 70-180g were purchased from the Veterinary Medicine unit of the University of Ibadan. They were housed in the experimental animal house, Department of Biochemistry, University of Ibadan. The animals were fed with commercial rat pellets (Vita-Feeds, Ibadan, Nigeria) and water *ad libitum.* The animals were allowed to acclimatize for two weeks and were randomly distributed into twelve (12) treatment groups of five (5) animals each for the study. The animals were safely maintained and humanely handled according to the approved protocols for the use of laboratory animal by the ethical committee of the University of Ibadan, Nigeria.

Animal Grouping and Treatment

INH was administered to the rats orally at doses extrapolated to their respective body weights.

Group I: Distilled water (negative control)

Group II: Sodium Arsenite (positive control)

Group III: INH 2.5mg/kg body weight

Group IV: INH 5.0mg/kg body weight

The grouping were in triplicate representing the durations of treatment of: 30, 60 and 90 days respectively.

INH was orally administered daily for the respective duration of treatment and animals were sacrificed at each treatment endpoint of 30, 60 and 90 days respectively. Blood glucose levels were monitored during the course of treatment with the aid of Fine-test™ Glucometer and test strips weekly. Animals were sacrificed at each treatment endpoint, by cervical dislocation and whole blood obtained by retro-orbital puncture. In addition the liver and femurs were harvested and processed for histological and micronuclei analysis.

Micronuclei Assay

Effects of INH on chromosomal aberration (clastogenicity) was evaluated in rat bone marrow cells using the method described by Heddle and Salmone and modified by Heddle *et al.* The animals were injected intraperitoneal with 0.04 % colchicine (1mL/100g body weight about 2hrs before sacrifice) and the bone marrow collected on clean slides. The slides were air-dried and pre-treated with May-Grunewald solution followed by staining with Giemsa solution. Scoring for the presence of micronucleated polychromatic erythrocytes (mPCEs) was performed under a light microscope with the aid of a tally counter [11].

Enzyme Assays

Whole blood collected from experimental animals were allowed to coagulate for about two hours after which they were centrifuged (3,000g, 4°C for 10 minutes) to separate cells from serum which was collected into clean Eppendorf tubes and immediately stored at -20°C until required. Serum level of hepatic transaminases: γ-GT was assayed with the reagent kit according to the method of Szasz [12]. Serum ALT and AST activities were also assayed according to the method of Reitman and Frankel [13]. Furthermore, serum urea and creatinine, albumin and total protein levels were assessed using standard kit. Antioxidant activities; glutathione-S-transferase (GST), catalase and superoxide dismutase (SOD) were also evaluated using methods described by Habig, 1974 and Mistra and Fridovich, 1972 [14, 15].

Histopathology

Harvested livers from experimental animals were weighed, and sections were processed, fixed in neutral formalin buffer, prior to histological analysis. Photomicrographs of processed liver section were taken using a Nikon E200 microscope at magnification X400.

UMU-Chromotest

INH genotoxicity was examined using the bacterial UMU-Chromotest assay Kit (EPBI, Ontario, Canada).

Statistical Analysis

Data analysis was carried out with the aid of GraphPad™ Prism analytical software employing the one-way Analysis of Variance (ANOVA) with the Statistical Package for Social Sciences (SPSS) software, version 17. Furthermore, comparison among different means was done with Duncan multiple range tests and statistical significance was set at $p<0.05$. Results were expressed as mean ± Standard deviation.

RESULTS

Administration of INH Cause a Decrease in Blood Glucose Level

Table **1**, shows a consistent reduction in blood glucose level in animals treated with INH (2.5 and 5.0mg/Kg) body weight treatment (30, 60 and 90 days). The decreases in blood glucose levels were statistically significant (*p<0.05*) relative to control groups.

Effect of Isoniazid (2.5 and 5mg/kg Body Weight) on Body Weight Change and Relative Liver Weight at Days 30, 60 and 90 Respectively

Body weight and liver weights of animals treated with INH for 30, 60 and 90 days duration are depicted in Table **2** a, b and c. There were observable reductions in percentage weight changes in the treatment group at both doses relative to control group. Furthermore, there were increases in liver and relative liver weight, across both treatment groups and the respective treatment durations. This pattern was not consistent, since there was a reduction at day 60 for the 5mg/kg body weight dose. The observed increases

Table 1: Blood Glucose Level in Rats Treated with Isoniazid (INH: 2.5mg and 5mg/kg Body Weight) for 30, 60 and 90 Treatment Days.

Blood Glucose Level (mg/dl)			
Groups	**30 days**	**60 days**	**90 days**
Control (-ve)	110±13	122±5.1	107±17
Control (+ve)	108±14	98±11	92±9.1
INH (2.5mg/kg)	76±23[a,b]	80±18[b]	68±28[b]
INH (5mg/kg)	85±11[b]	74±20[a,b]	66±18[a,b]

[a]Statistically significant when compared with positive control, [b]significant when compared with negative control, [a, b]significant when compared with both positive control and negative control at p<0.05>. All results are expressed as means ± SD, n=5 and p (<0.05).

Table 2: Body and Organ Weight Change in Rats Treated with Isoniazid (INH: 2.5mg and 5mg/kg Body Weight) for 30, 60 and 90 Treatment Days

Duration	Groups	IBW (g)	FBW (g)	WC (%)	LW (g)	RLW (%)
(A) 30 days	Control (-ve)	74	155	109.5	5.5	3.5±0.2
	Control (+ve)	98	166	69.4	6.5	3.9±0.32
	INH (2.5mg/kg)	124	179	44.5	6.1	3.4±0.19[a,d]
	INH (5mg/kg)	153	185	20.9	7.1	3.8±0.28[c]
(B) 60 days	Control (-ve)	106	241	127.4	8.2	3.4±0.21
	Control (+ve)	172	251	45.9	8.5	3.4±0.3
	INH (2.5mg/kg)	126	185	46.8	7.6	4.1±0.2[a,b, d]
	INH (5mg/kg)	156	223	42.9	5.7	2.6±0.05[a,b, c]
(C) 90 days	Control (-ve)	76	208	173.7	6.7	3.2±0.5
	Control (+ve)	100	243	143.0	8.3	3.4±0.19
	INH (2.5mg/kg)	106	245	131.1	9.1	3.7±0.28
	INH (5mg/kg)	113	230	103.5	9	3.9±0.53[b]

2A: [c]statistically significant when compared with the 2.5mg/kg INH; [a,d]significant compared with positive control and 5mg/kg INH.
2B: [a,b,c]statistically significant when compared with positive, negative controls and 2.5mg/kg INH treatment, [a,b,d]significant when Compared with positive, negative control and 5mg/kg INH treatment. **2C**: [b]statistically significant when compared with negative control.
All results are expressed as means ± SD, n=5 and p (<0.05). Abbreviations: Initial body weight (IBW); Final body weight (FBW); Body weight change (WC); Liver weight (LW); Relative liver weight (RLW); Isoniazid (INH).

Table 3: Effect of Isoniazid (INH: 2.5mg and 5mg/kg Body Weight) for 30, 60 and 90 Treatment Days on Serum Level of Hepatic Transaminases in Rats

Duration	Groups	AST (IU/L)	ALP (IU/L)	ALT (IU/L)	GGT (IU/L)
		A	B	C	D
30 days	Control (-ve)	4.9±0.89	82±1.4	6.6±1.2	2.0±1.0
	Control (+ve)	4.3±0.65	23±3.5	6.4±1.8	1.9±1.0
	INH (2.5mg/kg)	2.9±0.85[b]	83±20[a]	9.7±1.3[a,b, d]	3.1±0.6
	INH (5mg/kg)	3.7±1.2	85±13[a]	5.7±1.8[c]	1.6±0.2
60 days	Control (-ve)	4.5±0.58	61±4.5	7±2.7	2.7±0.5
	Control (+ve)	1.2±0.58	51±3.2	2.6±1.7	2.5±0.9
	INH (2.5mg/kg)	14±1.4[a,b, d]	82±5.3[a,b, d]	7.4±1.4[a]	7.2±1.2[a, b, d]
	INH (5mg/kg)	2.7±0.67[b,c]	67±2.8[a,c]	5.4±2.3	5.2±1.3[a,b, c]
90 days	Control (-ve)	5.1±1.5	48±5.8	4.7±0.58	3.0±0.6
	Control (+ve)	2.5±0.65	30±7.2	2.1±0.77	1.4±0.3
	INH (2.5mg/kg)	17±4.1[a,b]	131±17[a,b]	16±1.7[a]	17±1.6[a, b]
	INH (5mg/kg)	14±1.5[a,b]	125±13[a,b]	4.6±0.57[a]	16±3.2[a, b]

3A: [c], [b]statistically significant compared with negative control; [a, b]significant when compared with controls; [a, b, d]significant compared with controls and 5mg/kg INH treatment; [b, c] significant compared with negative control and 2.5mg/kg INH. **3B**: [a] Statistically significant compared with positive control; [b]significant compared with negative control; [a, b]significant compared with controls. [a, c]significant compared with positive control and 2.5mg/kg INH; [a, b, d]significant compared with controls and 5mg/kg INH. **3C**: [a]statistically significant compared with positive control; [c]significant compared with 2.5mg/kg INH; [a, b, d]significant compared with controls, and 5mg/kg INH. **3D**: [a, b]statistically significant compared with control; [a, b, c]significant compared with controls and 2.5mg/kg INH; [a, b; d]statistically significant compared with controls and 5mg/kg INH. All results are expressed as means ±S.D, n=5. Abbreviations: Aspartate amino transferase (AST); Alkaline phosphatase (ALP); Alanine amino transferase (ALP) and Gamma glutamyl transferase (GGT). Isoniazid (INH).

were insignificant (*p>0.05*) for the treatment groups except at days 60 and 90 (*p<0.05*). Significant differences were observed between the mean differences in liver weight between the two treatment doses.

Liver and Kidney Function Evaluation Following Prolonged Administration of INH in Wistar Rats

Hepatic transaminase in serum of rats exposed to long term INH treatment is shown in Table **3**(a-d) revealed an exaggerated response in the activities of these enzymes. Prolonged exposure to Isoniazid (INH) led to significant (p <0.05 elevations of (γ-GT, ALT, AST and ALP), which are markers of hepatotoxicity. However, there was an observable dose-dependent response in the transaminases levels in serum. Changes in serum urea level were significantly impacted by INH treatment (*p<0.05*) and creatinine as shown in Table **4** for both doses during the different treatment duration. Albumin level was significantly (*p<0.05*) increased across the duration of treatment for both doses, as well as total protein although albumin values at day 60 was not significantly impacted by treatment. Alterations in the activities of antioxidant markers; catalase Glutathione-S- Transferase (GST), and Superoxide dismutase (SOD) was observed; catalase and SOD activity was significantly (p<0.05) decreased, while GST was not affected significantly as shown in Tables **5**.

Histological Changes in Rat's Hepatocytes Following Prolonged Administration of INH

Histopathological results from examination of liver sections corroborated findings from hepatic transaminases in serum for both doses and the different treatment endpoints (Figure **2**) plates 1-3. INH treated groups showed portal and central venous congestion, fibrosis and periportal cellular infiltration. In addition to diffuse hydropic degeneration of hepatocytes which was mild, moderate and severe at days 30, 60 and 90 respectively. However there was severity of pathological hallmarks at the 2.5mg/kg body weight dose, which corroborates the dose-dependent elevation of serum transaminases.

Chronic INH Administration Induced Micronucleated Polychromatic Erythrocytes (mPCEs) Formation in Rats

Table **6** shows an increase (*p<0.05*) in micronucleated polychromatic erythrocytes (mPCEs) formation, in treated animals. These observations were visible irrespective of treatment duration and doses. However, the increases in mPCEs were observed to be dose-dependent as there was a higher induction for the 2.5mg/kg INH than in the 5mg/kg INH treated rats.

Table 4: Effect of INH Administration on Serum Urea and Creatinine, Albumin and Total Protein Level

Duration	Groups	Urea (µmoles/L)	Creatinine (mg/mL)	Albumin (ng/mL)	Total protein (mg/mL)
		A	B	C	D
30 days	Control (-ve)	32±2.7	0.27±0.054	3.1±0.7	5.9±0.65
	Control (+ve)	33±2.0	0.41±0.085	4±0.85	6.4±0.48
	INH (2.5mg/kg)	18±2.5[a, b, d]	1.2±0.28[a, b, d]	2.2±0.95[a]	3.7±0.58[a, b]
	INH (5mg/kg)	23±3.6[a, b, c]	0.16±0.068[ac]	1.9±0.59[a, b]	3.4±0.42[a, b]
60 days	Control (-ve)	28±1.8	0.56±0.34	1.5±0.37	2.6±0.97
	Control (+ve)	6.1±1.7	0.4±0.071	1.9±0.38	2.6±0.73
	INH (2.5mg/kg)	29±3.0[a, d]	0.9±0.56[d]	2.5±0.41[b]	3.1±0.85
	INH (5mg/kg)	21±5.9[a, b, c]	0.24±0.091[c]	2.4±0.48[b]	2.9±0.73
90 days	Control (-ve)	25.1±1.5	0.47±0.084	1.6±0.28	3.4±0.28
	Control (+ve)	22.5±0.65	0.58±0.058	1.8±0.17	1.9±0.19
	INH (2.5mg/kg)	17±4.1[a,b]	0.47±0.054[a]	1.4±0.096[a]	2.6±0.43[a, b]
	INH (5mg/kg)	24±1.5[a,b]	0.42±0.045	1.3±0.23[a, b]	2.2±0.039[b]

4A: [a, d]statistically significant compared with positive control and 5mg/kg INH treatment; [a, b, c]significant compared with control and 2.5mg/kg INH; [a, b, d]significant compared with control and 5mg/kg INH treatment. 4B: [a]significant compared with positive control; [c]significant compared with 2.5mg/kg INH; [d]significant compared with 5.0mg/kg INH; [a, c]significant compared with positive control and 2.5mg/kg INH; [a, b, d]significant compared with controls and 5mg/kg INH treatment. 4C: [a]significant compared with positive control; [b]significant compared with negative control; [a, b]significant compared with controls. 4D: [b]significant compared with negative control; [a, b]significant compared with both controls. All results are expressed as means ±S.D, n=5. Abbreviation: Isoniazid (INH).

Table 5: Effect of INH Treatment on SOD, GST and Catalase Activity

Duration	Groups	SOD (Units/g Tissue)	GST (µmoles/min/ mg protein)	Catalase (µmoles/mg protein)
		A	B	C
60 days	Control (-ve)	0.42±0.38	43±20	47±12
	Control (+ve)	1.9±0.61	48±1.8	273±64
	INH (2.5mg/kg)	0.29±0.13[a]	6.5±3.2[ab]	227±21[b]
	INH (5mg/kg)	0.21±0.093[a]	13±9.6[ab]	180±26[ab]

6A: [a]Statistically significant compared with positive control; 6B: [a, b]significant compared with controls; 6C: [b]significant compared with negative control; [a, b]significant compared with controls. All results are expressed as Means ±S.D, n=5; p-values are set at (<0.05). Abbreviation: Superoxide dismutase (SOD), Glutathione-S-transferase (GST).

Table 6: Treatment with Isoniazid Induced Micronucleated Polychromatic Erythrocytes (mPCEs) in Rats

Treatment Duration	Groups	mPCEs Induction
30 days	Control (-ve)	0.17±0.1
	Control (+ve)	0.82±0.63
	INH (2.5mg/kg)	3.83±1.88[b]
	INH (5mg/kg)	3.8±1.58[b]
60 days	Control (-ve)	1.75±0.19
	Control (+ve)	3.14±0.69
	INH (2.5mg/kg)	3.28±0.82[b]
	INH (5mg/kg)	2.88±0.26[b]
90 days	Control (-ve)	1.0±0.81
	Control (+ve)	14.5±1.29
	INH (2.5mg/kg)	2.8±0.63[b]
	INH (5mg/kg)	3.0±0.64[b]

[b]Statistically significant compared with negative control. Results are expressed as means ±S.D, n=5.
p-values are set at (<0.05). Abbreviation: micronucleated polychromatic erythrocytes (mPCEs).

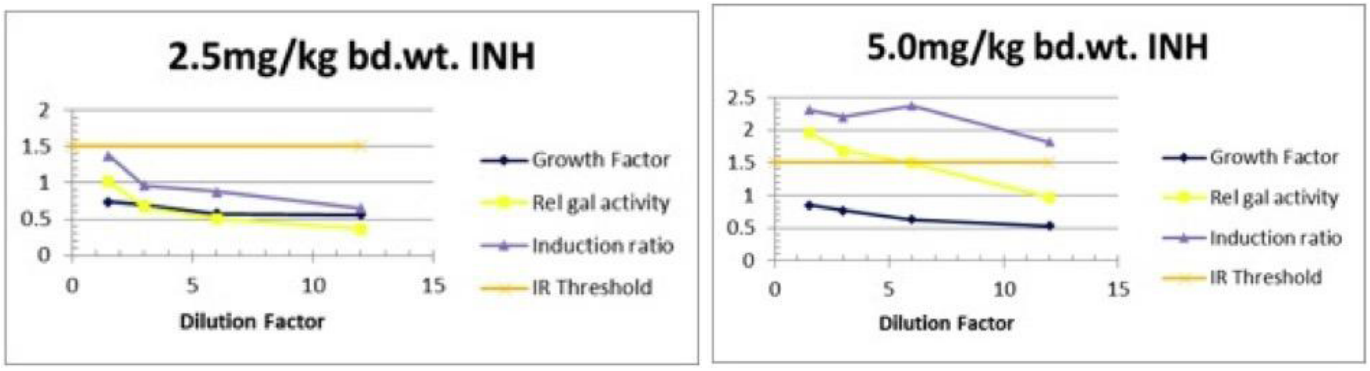

Figure 1: Determination of Isoniazid-induced genotoxicity at 2.5 and 5mg/kg by UMU Chromotest.

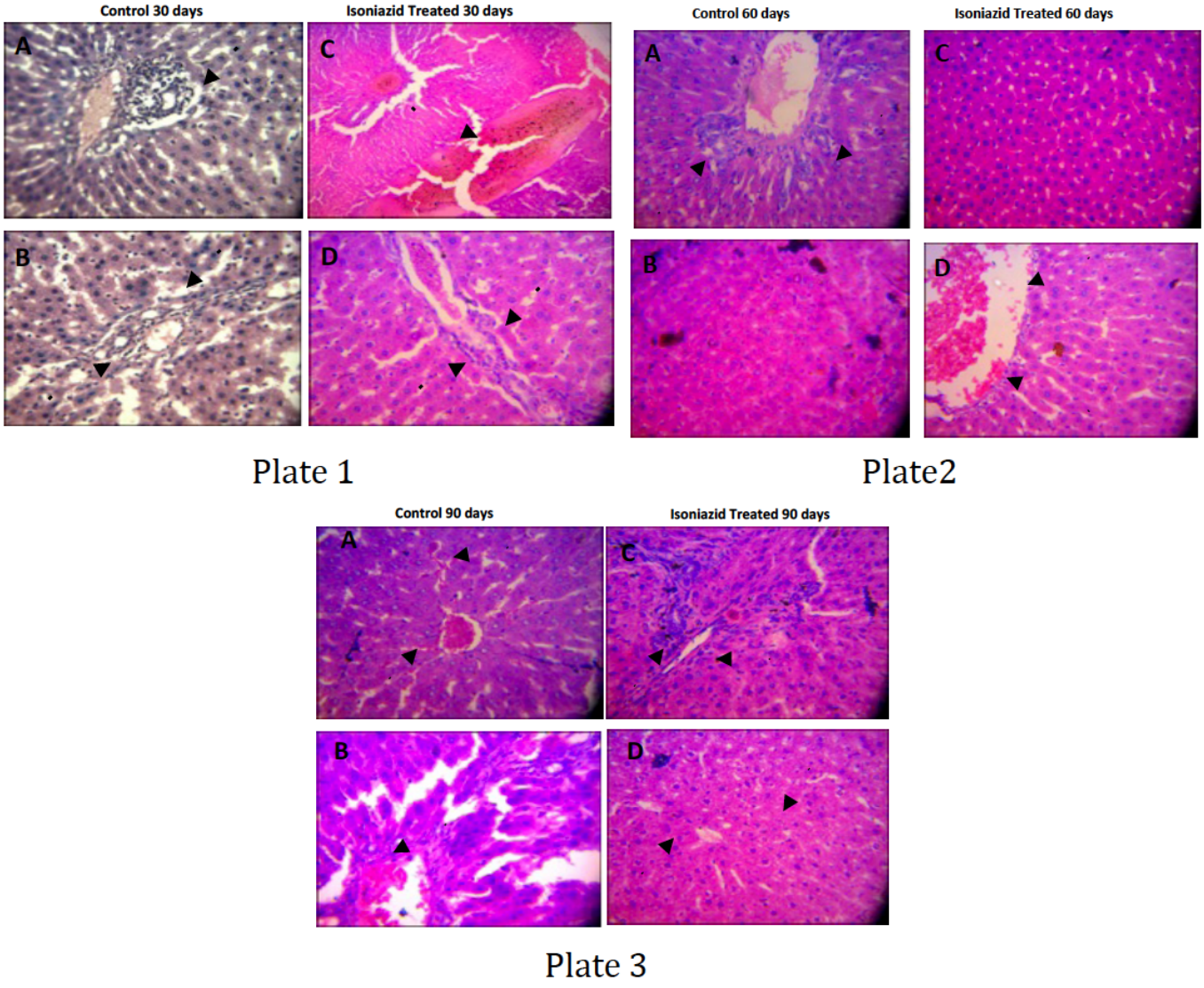

Figure 2: Photomicrograph of rats' livers treated with INH for 30, 60 and 90 days. Plate 1: (A) negative control exhibiting moderate congestion and cellular infiltration by macrophages with limited neutrophils at the portal area. (B) Positive control (sodium Arsenite) with mild portal cellular infiltration. (C) Liver section of INH (2.5mg/kg) treated animals showing severe portal and central venous congestion and (D) Liver section of INH (5mg/kg) with mild portal congestion and periportal cellular infiltration. Plate 2: A (-ve) and B (+ve) controls with moderate congestion and cellular infiltration respectively, (C) INH (2.5mg/kg) with limited lesion (D) INH (5mg/kg) severe central venous congestion. Plate 3: (A) Section of control (-ve) showing very mild portal and sinusoidal congestion. (B) (+ve control) showing mild to moderate periportal cellular infiltration. (C) INH (2.5mg/kg) moderate to severe cellular infiltration and fibrosis at the portal area (D) INH (5mg/kg) treated rats showing mild diffuse hydropic degeneration of hepatocytes. All sections were paraffin embedded tissue and stained with H & E, magnification: X400.

Evaluation of Genotoxicity of the Test Substance at both Treatment Doses

INH was genotoxic at 5mg/kg body weight in the bacterial UMU chromotest, whereas treatment with 2.5mg/kg body weight dose was not genotoxic. This was evaluated using the UMU induction ratio threshold (UMU-IR) set at 1.5. The 2.5mg and 5mg/kg body weight doses have an IR threshold below and above 1.5 respectively as shown in Figures **1**.

DISCUSSION

Isoniazid (INH) is the primary drug widely used as a first-line treatment regimen for the management both latent and active infection with tuberculosis. Hepatic metabolism of INH, release metabolites, which include hydrazine, acetyl hydrazine, generating free radicals which alters hepatic pro-oxidant, involve in inducing liver damage in rats [16].

Alteration in the pro-oxidant – antioxidant balance to favor pro-oxidants results in oxidative stress and a reduction in the endogenous free radical scavenging potentials GST, catalase, and SOD as a result of increase in the activity of free radicals [17]. In addition, damages to cellular macromolecules and deoxyribonucleic acid (DNA) through the activities of the free radicals released as a by product of metabolism [18], can be exacerbated.

However, isoniazid usage as a first-line choice in the management of tuberculosis requires oral administration of a total daily dose of 5mg/kg with a maximum of 300mg for the first two months. An adjusted dose of INH is continued for a 'continuation phase of treatment'' of 3-9 months depending on the type of infection (sensitivity of the bacilli); whether latent or active.

However, INH therapy over this long period of time can account for free radicals generation therefore altering antioxidant levels and constitutively causing damages to cellular macromolecules especially DNA, hepatocyte antioxidant activity, which helps maintain cellular integrity. This may result in altered hepatic functions, formation of DNA adduct as a result of inadequate DNA repair capacity, necrosis and ultimately cell death releasing more biochemical, which may have implication in hepatocarcinogenesis [19].

Free radicals damage the integrity of cellular membrane and also induce direct or indirect lipid peroxidation. Furthermore, altering hepatocytes function as the site of primary INH metabolism due to the deteriorated cellular membranes and loss of cellular integrity [20]. This could further result to liver injury, atherosclerosis, and damage to the kidney notably in higher animals [21]. Reduction in animals' body weight observed with INH dosing (INH: 2.5 and 5mg/kg) buttresses finding of observable weight loss at the end of the dosage regimen. Attributed to loss of appetite exhibited by the treated animals as a result of the drug-induced stress. In addition, reduction in blood glucose level during the duration of INH administration (30, 60 and 90 days), may be as a result of the increased glucose utilization by cells which tends to be damaged (abnormal) as a result of consistent INH administration, similar to the Warburg effect in which abnormal cells (cancerous) greatly utilize glucose for their metabolism and ATP production at the expense of normal cells [22]. INH hepatotoxicity was further demonstrated by increases in hepatic transaminases in serum. These is indicative of INH-induced liver damage, and diminished hepatocytes integrity causing leakage into the blood [23]. This observation was consistent for both doses (2.5 and 5mg/kg B.W) at days 30, 60 and 90 respectively, further confirming INH hepatotoxicity, which was consistent with previous studies although it was carried out for duration of 21 days [24].

However, there was a much higher elevation in the level of these marker enzymes for the 2.5mg/kg dose compared to the 5mg/kg B.W dosage, a pattern that was consistent for the enzymes and the respective duration of administration. This observation might be as a result of hepatic adjustment and reduced sensitivity to INH at the lower dose (2.5mg/kg B.W) administered compared to the higher dose (5mg/kg B.W) over a period of time.

Administration of INH at both 2.5 and 5mg/kg B.W resulted in a decline in the levels of Albumin and total protein thereby impairing liver function [25].

Serum urea and creatinine were assessed in this study to determine renal function, elevated serum levels of urea and creatinine are indicative of renal dysfunction [26]. This was observed for creatinine after INH administration for both doses (2.5 and 5mg/kg) at days 30, 60 with a decline at day 90. This could be as a result of adaptive mechanisms in which the body system adjusts to contain INH toxicity during a lengthy course of administration at day 90. Although, was not the case with urea whose level increased after 60 days of treatment, albeit insignificantly. The observed changes in urea level maybe as a result of liver and kidney dysfunction.

Antioxidants such as SOD and catalase play important roles in scavenging free radicals upon their appearance *in vivo* thus playing an important defensive roles against their oxidative stress inductive ability [27]. Our results however, indicate idiosyncratic alterations in the activities of antioxidants; SOD, catalase and GST upon administration of INH at both 2.5 and 5mg/kg body weight. There was reduced catalase and GST activity (although insignificant), compared to the control animals. However, there was an elevation in the activity of SOD. These alterations may indicate the role of INH in inducing oxidative stress, which is in tandem with previous work done [23].

Furthermore, the result of the present study clearly shows that both INH doses administered (2.5 and 5mg/kg body weight) caused mPCEs formation in the bone marrow cells at days 30, 60 and 90 respectively dose-dependently, indicative of the potential genotoxic effect of prolong exposure to INH.

Furthermore, the UMU chromotest was used to ascertain the genotoxicity of INH using the Umu Induction Ratio (Umu IR) and growth factor (GF): indication for a test substance to be considered genotoxic, it must exhibit Umu IR > 1.5 and a growth factor > 0.5. The present study indicated a Umu IR < 1.5 and G.F. = 0.5 (at INH 2.5mg/kg), and an Umu IR >1.5 and G.F >0.5 (at INH: 5.0mg/kg). Confirming the dose-dependent genotoxicity of INH in the UMU bacterial genotoxicity assay.

Histopathological examination revealed mild to severe portal and central venous congestion, cellular infiltration and fibrosis on the liver section of INH intoxicated animals at both doses of INH (2.5 and 5mg/kg). These observations taken together indicate that prolonged exposure to INH can result in oxidative stress especially in hepatocyte the primary locus of drug metabolism. Impaired clearance of reactive oxygen and nitrogen species may play a role in the damage of cellular macromolecules including dysfunction protein formation and mutation in DNA that may be relevant in cellular transformation and hepatocarcinogenesis. Steps should be taken to mitigate the damage caused by therapeutic INH, while safer alternatives to INH are being developed to safeguard human and public health.

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ABBREVIATIONS

INH = Isoniazid

mPCEs = micronucleated polychromatic erythrocytes

ALT = alanine amino transferase

AST = aspartate amino transferase

γ-GT = gamma glutamyl transferase

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