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Research Article
Comparative *in vivo* Evaluation of Rat Liver and Kidney Histomorphology Following Treatments with Doxorubicin, Acetaminophen and Anti-Tubercular Drugs

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Abstract

Background and Objective: Doxorubicin (an anti-cancer drug), acetaminophen (a non-steroidal anti-inflammatory drug) and first-line anti-tubercular drugs (rifampicin, isoniazid and pyrazinamide) have been used to induce organ toxicity in animal experiments. Despite many respective reports on their organ toxicities, there is a lack of literature on studies aimed at comparing the pattern of injuries exerted by these drugs on the microanatomical structures of the liver and kidney tissues *in vivo*. The present study, for the first time, demonstrated and compared the effects of doxorubicin (DOX), acetaminophen (APAP) and first line anti-tubercular drugs (ATB) on body weights, relative organ weights and light microscopical features of the liver and kidneys of albino rats. **Materials and Methods:** Male albino rats in respective groups were treated with intra-peritoneal doses of DOX (20 mg kg^{-1}), oral doses of APAP (500 mg kg^{-1}) and ATB drugs (200 mg kg^{-1} rifampicin+150 mg kg^{-1} isoniazid+500 mg kg^{-1} pyrazinamide), once daily for 7 days. The effects of the treatments on relative organ weights and histopathology of the liver and kidney were determined. **Results:** The body weight loss was observed in all treatment groups when compared to the control ($p<0.05$). The liver index decreased in DOX and APAP groups while the kidney index increased in DOX and ATB groups when compared to the control ($p<0.05$). Histopathological studies revealed characteristically distinctive patterns of injuries in the liver and kidney of rats in the three different groups with the DOX group showing the most profound deleterious changes. **Conclusion:** Findings obtained from this study provide the light microscopical *in vivo* evidence of hepatotoxicity and nephrotoxicity by the DOX, APAP and ATB after a short-term administration, with DOX exerting the most profound microanatomical alterations in the liver and kidney tissues.

Key words: Doxorubicin, acetaminophen, antitubercular drugs, hepatotoxicity, nephrotoxicity, histopathology, troglitazone

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The liver is a critical organ of the body which plays a major role in metabolism with various functions including detoxification, regulation of glycogen storage, production of bile, plasma protein synthesis, decomposition of red blood cells and hormone production. The kidneys, on the other hand, are responsible for blood filtration to remove waste products of metabolism. Its main function is to regulate the balance of blood electrolytes and maintain pH homeostasis.

The organs of the body most that are most susceptible to organ toxicity are the liver and kidney[1] because they serve as sites for metabolic breakdown of toxins and filtration respectively. The repeated use of drugs, even within therapeutic ranges are prone to exert many side effects.

This invariably causes the accumulation of the drug and its potentially toxic metabolites in the body tissues/organs and eventually lead to organ damage. Hepatotoxicity and nephrotoxicity are terms used to define the states of toxic damage to the liver and kidney, respectively. Drug-related hepatotoxicity can be mediated via many different mechanisms either by the toxic metabolites eliciting an immune system or by acting directly on the liver cells to cause intracellular stress[2]. Nephrotoxicity occurs when there is inefficient kidney-specific detoxification and excretion as a result of injuries exerted by exogenous or endogenous toxicants[3]. Many drugs have been associated with drug-induced hepatotoxicity and/or nephrotoxicity such as troglitazone, bromfenac, hallothane, cystatin, methotrexate, acetaminophen, doxorubicin and anti-tubercular drugs among others[4-6].

For more than two decades, researchers have continued to search for agents from natural sources with potential organ-protective capabilities and thus have employed some drugs as toxicants in order to induce organ damage in experimental animal models[6,7]. Doxorubicin, acetaminophen and anti-tubercular drugs are commonly used as toxicants and their effects on tissues have been documented differently[8-12].

However, a careful literature search revealed that a study comparing the *in vivo* effects of the drugs on both the liver and kidney microanatomical structures is lacking. There is a need to demonstrate and identify the pathological lesions that these drugs will exert at the microanatomical level when administered for a short duration as it will enable researchers to make the proper adjustment on the dose of treatment or on the toxicant of choice. Hence, this study was therefore, designed to demonstrate and compare the patterns of injuries induced by short-term administration of doxorubicin, acetaminophen and anti-tubercular drugs in albino rats.

MATERIALS AND METHODS

Study area and duration: The study was carried out at Panacea Diagnostic and Research Laboratories and Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria, Enugu Campus, Nigeria between March, 2016 and August, 2017.

Laboratory animals: Sixteen albino male Wistar rats (weighing 130-160 g) were used for the experiment. They were obtained from the Animal House of the University of Nigeria, College of Medicine, Enugu Campus and were housed at Panacea Diagnostic and Research Laboratories under standard conditions of temperature (25±2°C) and relative humidity (40-70%) with lighting of 12 hrs light/dark cycle. The animals were given free access to standard rat chow (Standard Top® feed) and tap water. Animals were acclimatized to laboratory conditions for 7 days before conducting the experiments. Animal housing and handling were performed in strict compliance with institutional and international guidelines for the care and use of animals in Scientific Research[13].

Experimental design: Sixteen rats were divided into four groups of four rats each, labeled I to IV and were treated thus:

- **Group I (control):** Received only water
- **Group II:** Received 20 mg kg^{-1} b.wt., of doxorubicin (DOX) intra-peritoneally (i.p.)
- **Group III:** Received 500 mg kg^{-1} b.wt., of acetaminophen (APAP) by oral gavage
- **Group IV:** Received anti-tubercular drugs (ATB) (200 mg kg^{-1} b.wt., of rifampicin (RMP)+150 mg kg^{-1} b.wt., of isoniazid (INH)+500mg kg^{-1} b.wt., of pyrazinamide (PZM)) by oral gavage

All drug administrations were given once daily for 7 days.

Body weight measurements: The rats were weighed before acclimatization, at the commencement of the experiment (day 0) and at the end of the study (day 7).

Animal sacrifice, necropsy and organ-to-body weight index: At the end of the study (day 8), the rats were all sacrificed under light chloroform anesthesia. Each rat was dissected for the excision of the liver and kidneys to be made before necropsy. The organs were weighed using a sensitive analytical balance for the determination of the organ-to-body

weight index otherwise known as relative organ weights. The calculation for each rat was achieved as the ratio of organ weight and the animal's body weight at the end of the study, multiplied by 100.

Histopathological studies:

- **Tissue fixation:** The tissues were immediately rinsed with normal saline before placing them in their respective group containers bearing 10% formol-saline for further histological processing. Fixation lasted for 24 hrs before they were subjected to stages in histological processing
- **Tissue processing:** Dehydration in ascending grades of alcohol, clearing in changes of xylene, infiltration and embedding with paraffin wax in tissue cassettes as described by Suvarna *et al.*[14] were carried out on the tissues using the automatic tissue processor. The slicing of the tissues (sectioning) was achieved using a Rotary microtome (Hertz 150 rotary Cambridge model). Sections were then stained according to haematoxylin and eosin (H&E) technique as described by Dey[15]

Microscopy and photomicrography: For microscopical examination, 10x and 40x objectives of Olympus™ Binocular microscope with an inbuilt lighting system were used. Areas bearing the most prominent lesion were photomicrographed using a digital microscope eyepiece camera (AmScope MU300 series Model).

Statistical analysis: Statistical analysis was performed using the statistical computer software program known as Statistical Package for the Social Sciences (SPSS) Software Version 23. Results obtained were presented as Mean±Standard Error of the Mean (SEM). To determine the main effects on treatment groups, the One-way Analysis of Variance (ANOVA) was used, while the student's t-test and Tukey's *post hoc* test were used for multiple comparisons. The p-values less than 0.05 were considered significant.

RESULTS

Body weight changes: The mean body weights of rats taken before acclimatization, at the start and end of the study, were shown in Fig. 1a, whereas, Fig. 1b represented the body weight differences by the end of the study. All the rats increased in body weight after the 7 day acclimatization period but after treatments with the drugs, a statistically significant decrease ($p<0.05$) in body weights of rats in DOX, APAP and ATB-treated groups was observed when compared with the normal control. Body weight loss was most profound in DOX-treated rats with a difference of -52.50±4.79 g, followed by APAP (-29.00±3.19 g) and then ATB (-18.00±0.58 g) groups. However, the only group I (Control) rats increased in body weight (6.50±1.32 g) by the end of the study (Fig. 1b).

Organ indices: The effect of the treatments on liver and kidney indices were presented in Fig. 2. A significant decrease ($p<0.05$) in the liver index was observed in DOX (3.42±0.01) and APAP (4.06±0.23) -treated groups when compared to the control (5.20±0.11). Total kidney index revealed a statistically significant increase ($p<0.05$) in DOX (1.05±0.01) and ATB (1.302±0.04) treatment groups when compared to the control (0.89±0.03).

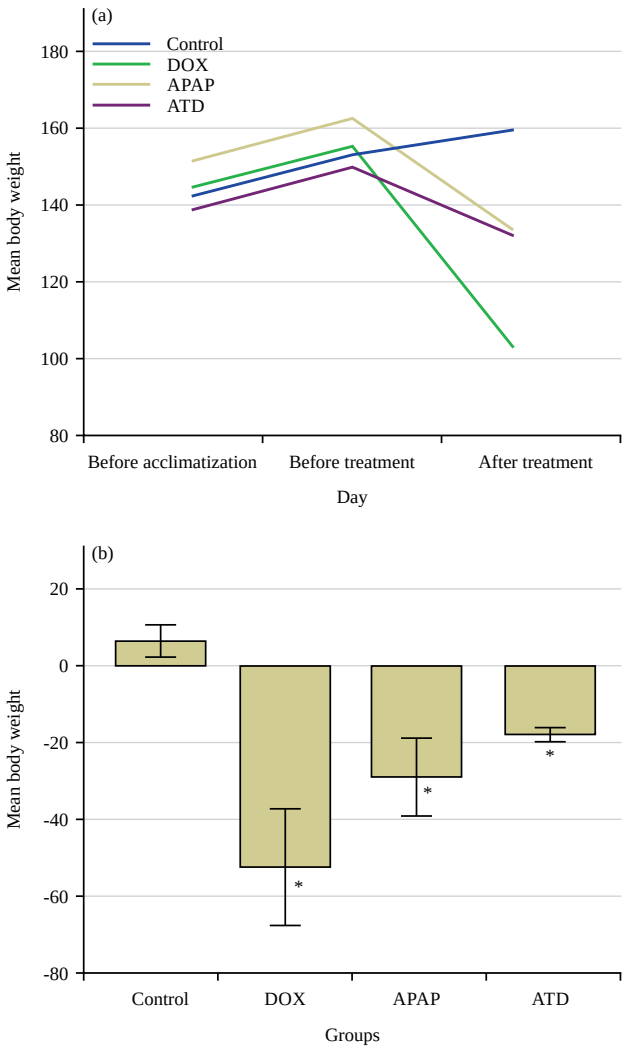

Fig. 1(a-b): Effect of treatments with doxorubicin (DOX), acetaminophen (APAP) and anti-tubercular drugs (ATB) on the (a) Rats' body weights and (b) Body weight differences at the end of the experiment
*p<0.05 when compared to control

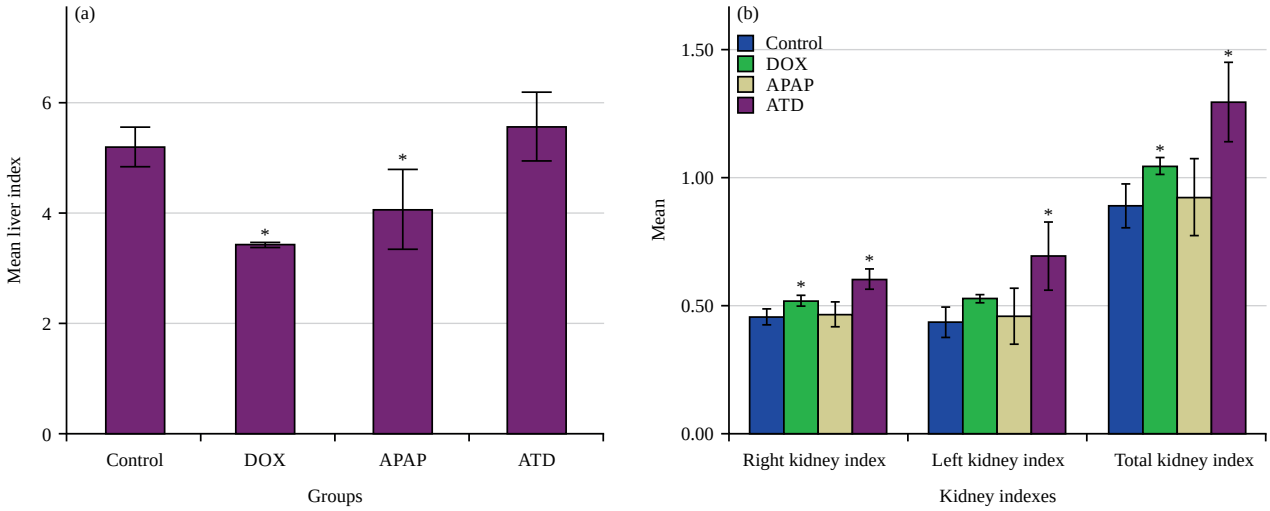

Fig. 2(a-b): Effect of treatments with doxorubicin (DOX), acetaminophen (APAP) and anti-tubercular drugs (ATB) on (a) Relative liver and (b) Relative kidney weights

*p<0.05 when compared to control

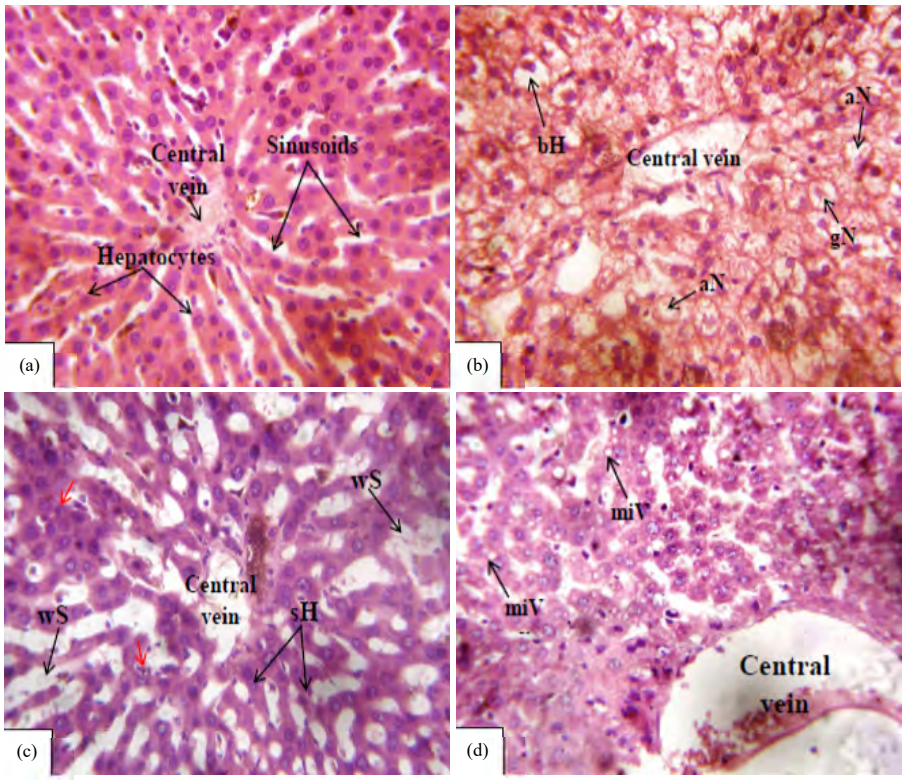

Fig. 3(a-d): Photomicrographical illustration of the central vein and centrilobular features in liver sections from rats in (a) Control and (b-d) Treatment groups, (a) Control rat liver showing normal histoarchitecture of the centrilobular region of the hepatic tissue, (b) Liver section of rat treated with 20 mg kg^{-1} b.wt., of doxorubicin showing evidence of tissue degeneration, (c) Liver section of rat treated with 600 mg kg^{-1} b.wt., of acetaminophen showing markedly shrunken centrilobular hepatocytes (sH) with the resultant widening of sinusoidal spaces (wS) and (d) Liver section of rat treated with anti-tubercular drugs showing central vein rupture, hepatocyte degeneration and vacuolation

bH: Hepatocytes appear ballooned, gN: ghost cells, aN: Absence of nucleus, miV: microvesicles, maV: macrovesicles and Stain: H&E/Mag.: x400

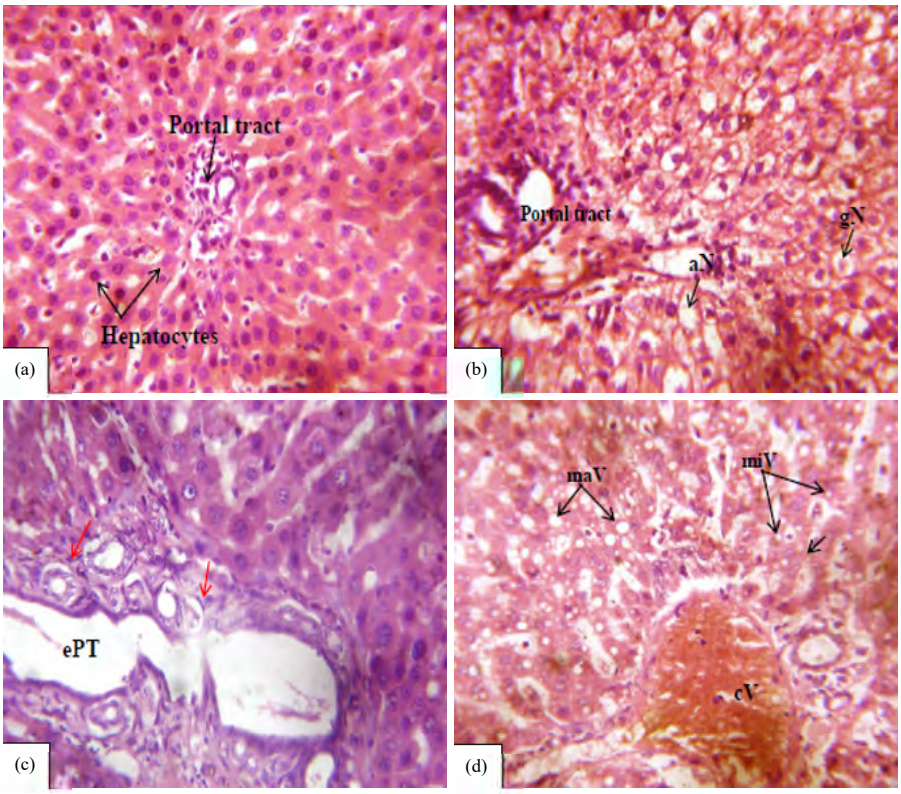

Fig. 4(a-d): Photomicrographical illustration of the portal tract and periportal features in liver sections from rats in (a) Control and (b-d) Treatment groups, (a) Control rat liver showing normal histoarchitecture of the periportal region of the hepatic tissue, (b) Liver section of rat treated with 20 mg kg^{-1} b.wt., of doxorubicin, (c) Liver section of rat treated with 600 mg kg^{-1} b.wt., of acetaminophen and (d) Liver section of rat treated with anti-tubercular drugs

bH: Ballooned hepatocytes, gN: Ghost cells and aN: Absence of nucleus, ePt: Enlarged portal tract, Red arrows: Inflammatory cellular infiltration, cV: Congested vessel, miV: microvesicles, maV: macrovesicles and Stain: H&E/Mag.: x400

Histopathological findings

Effects of the treatments on liver histomorphology: The pictorial illustrations revealing the features observed after treatments with DOX, APAP and ATB on the liver histomorphology of rats are shown in Fig. 3 and 4. The central vein and other features at the centrilobular region were shown in Fig. 3, while Fig. 4 depicted the portal tracts and periportal features.

In the control group, the histoarchitecture of the central vein, sinusoidal spaces, portal tract and hepatocytes appear normal (Fig. 3-4a). Comparison of the pathological alterations upon drug treatments for 7 days revealed significant effects of each of the drugs on the hepatic tissue. The central veins in all the treatment groups appear fairly intact except for the ATB group (Fig. 3d) with a ruptured central canal.

Regarding the hepatocytes, DOX caused ballooning degeneration and the presence of ghost cells (Fig. 3-4b), while ATB caused fatty degeneration of the hepatocytes as evidenced by the presence of vacuolations (Fig. 3-4d) at both centrilobular (Fig. 3) and periportal regions (Fig. 4). The APAP increased sinusoidal spaces and shrunk the hepatocytes only at centrilobular regions (Fig. 3c) whereas, periportal hepatocytes appeared unaffected (Fig. 4c).

For changes in the portal tracts (Fig. 4), profound alterations were noted upon treatments with APAP and ATB. Infiltration of inflammatory cells and enlargement of the portal tracts were the notable changes with APAP treatment (Fig. 4c), while vascular congestion was observed with ATB treatment (Fig. 4d).

Effects of the treatments on kidney histomorphology: The histomorphological changes in the kidney sections following the drug treatments were shown in Fig. 5 and 6, depicting changes within the cortex and medulla, respectively. In the control group, normal glomerulus, convoluted tubules, blood vessels, Bowman's capsule and space and medullary tubules (collecting ducts and loops of Henle) were observed (Fig. 5-6a).

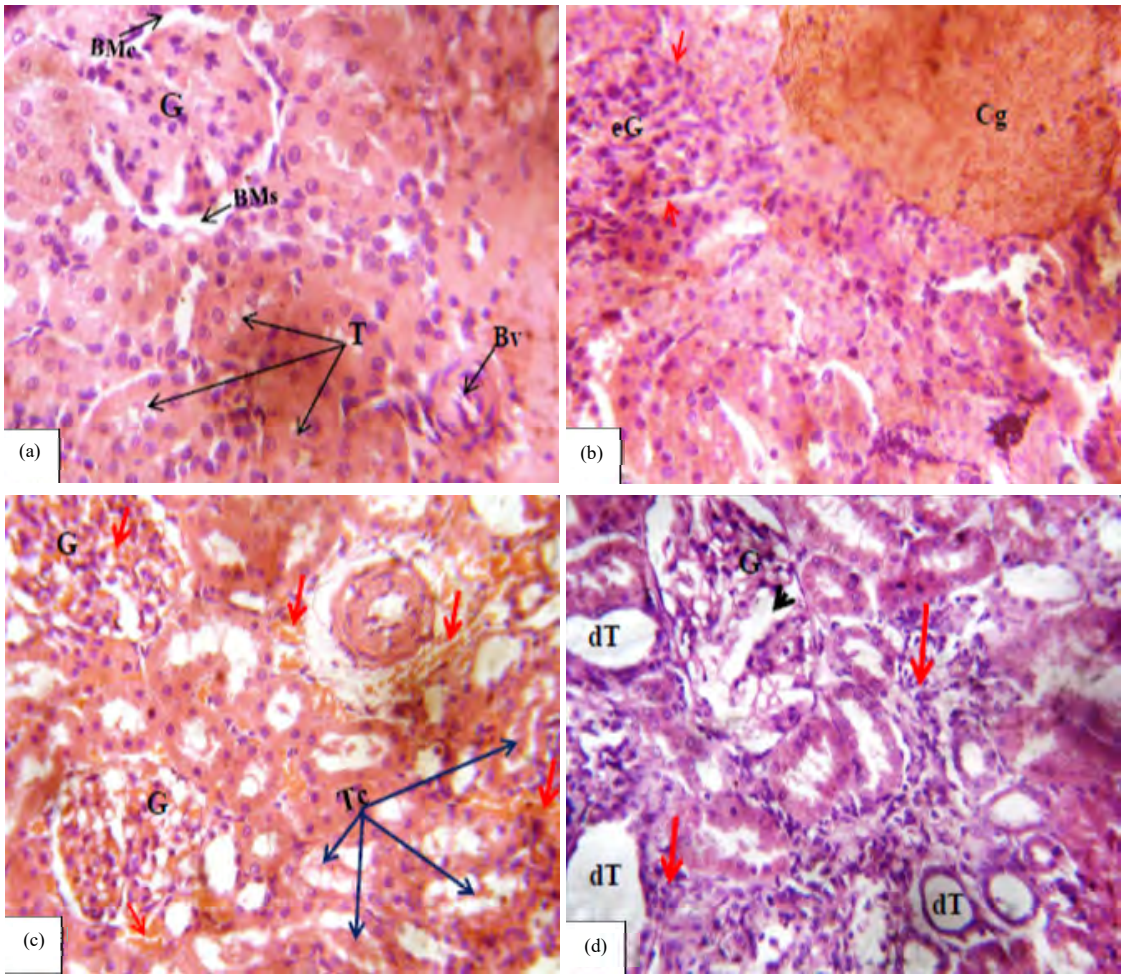

Fig. 5(a-d): Photomicrographical illustration of the cortex of kidney sections from rats in (a) Control and (b-d) Treatment groups, (a) Control rat kidney showing normal histoarchitecture of the cortex, (b) Cortical kidney section of rat treated with 20 mg kg^{-1} b.wt. of doxorubicin, (c) Cortical kidney section of rat treated with 600 mg kg^{-1} b.wt., of acetaminophen and (d) Cortical kidney section of rat treated with anti-tubercular drugs showing evidence of marked renal injury

(a) G: Glomerulus, T: Convoluted tubules, Bv: Blood vessels, Bmc: Bowman's capsule and Bms: Bowman's space, (b) eG: Enlarged glomerulus, Cg: Evidence of congestion and Red arrows: Bowman's capsule, (c) G: Glomeruli, Red arrows: Presence of casts in dilated cortical tubules and evidence of haemorrhage and (d) Arrowheads: Histological profile of the tissue reveals lobulation and hypocellularity of the glomerulus (G), Red arrows: Most tubules appear necrotic and the presence of inflammatory cellular infiltration is observed. Some tubules are dilated (dT) and present shrunken epithelia and Stain: H&E/Mag.: x400

For changes observed in the cortex, DOX and APAP caused an enlargement of the glomeruli (Fig. 5b-c, respectively) while A TB caused lobulation and hypocellularity of the glomeruli (Fig. 5d). Additionally, vascular congestion and tuft adhesion were noted with DOX treatment while haemorrhage was observed with APAP treatment. Intact cortical tubules are noted with DOX (Fig. 5b), however, tubular casts are seen in the APAP group (Fig. 5c) while marked inflammatory cellular infiltration, tubular degeneration and necrosis are observed in the ATB group (Fig. 5d).

In the medulla, the presence of inflammatory cellular infiltration was noted in kidney sections from DOX and ATB groups, although this feature was moderate with ATB treatment (Fig. 6d) and mild in the DOX group (Fig. 6b). Sloughed tubules were observed in the APAP group (Fig. 6c) whereas dilated tubules with shrunken epithelia were noted with ATB treatment (Fig. 6d).

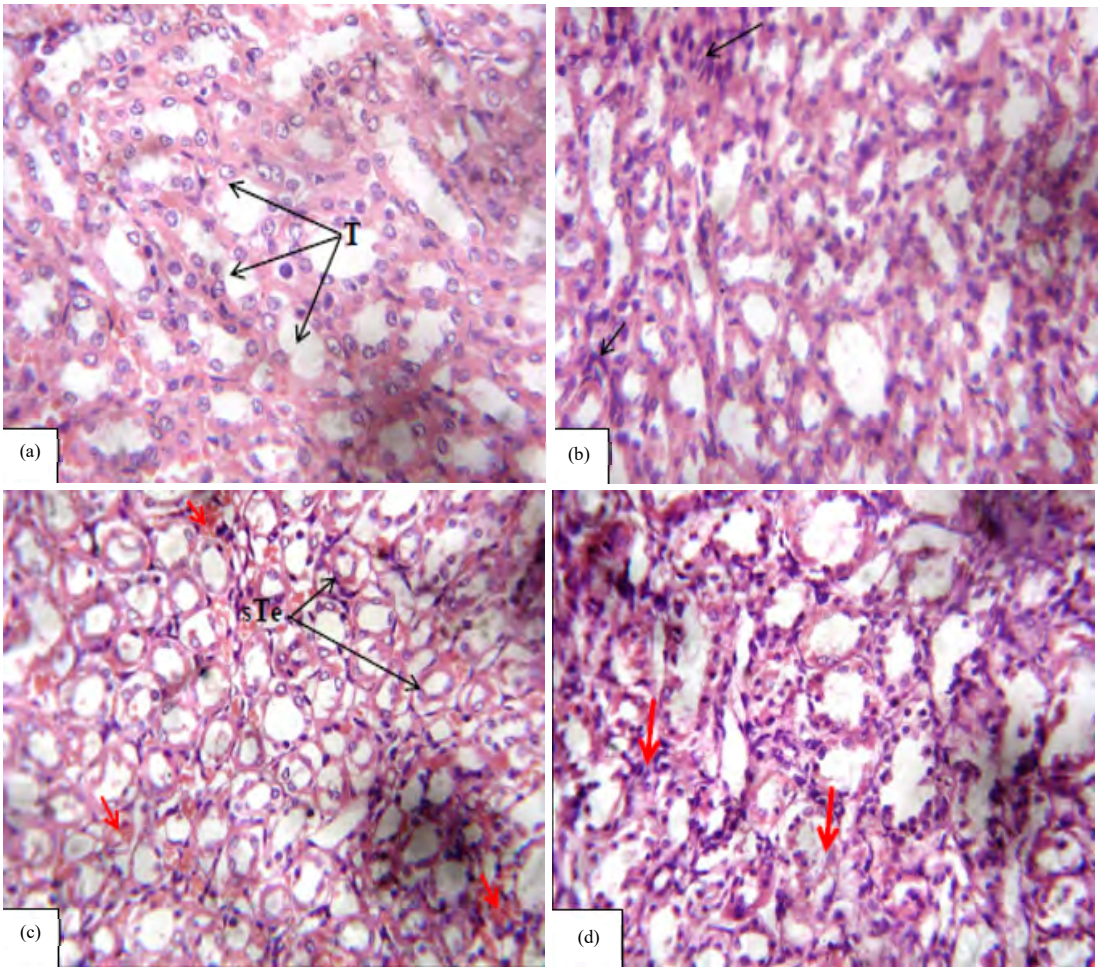

Fig. 6(a-d): Photomicrographical illustration of the medulla of kidney sections from rats in (a) Control and (b-d) Treatment groups, (a) Control rat section showing normal histoarchitecture of the renal medulla, (b) Kidney section of rat treated with 20 mg kg^{-1} b.wt., of doxorubicin, (c) Kidney section of rat treated with 600 mg kg^{-1} b.wt., of acetaminophen and (d) Kidney section of rat treated with anti-tubercular drugs

(a) Medullary T: Tubules (including the collecting ducts and loops of Henle) appear normal, (b) Black arrows: Mild inflammatory cellular infiltration, (c) sTe: Sloughed tubular epithelia, (d) Inflammatory cellular infiltration (Red arrows) dT: Tubules that are dilated and present shrunken epithelia and Stain: H&E/Mag.: x400

DISCUSSION

Although various studies have documented the toxicities of doxorubicin, acetaminophen and first-line anti-tubercular drugs (rifampicin, isoniazid and pyrazinamide) using animal models, the present study, however, represented the effects of these drugs comparatively, especially as it relates to histopathological alterations. Intoxication of rats with these drugs in this work was observed to produce profound effects on the body weight, organ indices and histomorphology of the liver and kidneys of treated rats.

The improved body weight of rats during the acclimatization period suggests that the animals were living in a controlled environment with standard living conditions as well as improved feeding with the standard diet. However, upon treatment with the drugs, marked body weight loss, even below their previous weights at the start of the experiment, was observed in all treated animals. It is well known that changes in body weight serve as a sensitive indicator for toxic agents and hence it's conventional use in predicting the toxicity of such substances[16]. The most profound loss of body weight was observed with DOX treatment. Severe weight loss, muscle atrophy and conditions similar to cachexia have been attributed to DOX treatment in previous studies[17,18]. This loss of body weight may be due to decreased appetite or a direct/indirect effect on the metabolic

rate. Considering the relative organ weight, which is an index used to estimate organ weight in relationship with the body weight of the animal[19], the marked changes observed in the present study could be attributed to the significant decrease in body weight of the treated rats. The results from organ weight measurements are often difficult to interpret especially because the changes observed may be due to chemically-induced organ damage or changes in the overall body weight[19].

The hepatotoxicity established by three-drug treatments in the present study revealed different patterns of injury as it pertains to hepatic cells, central vein and portal tracts. Doxorubicin (DOX) was observed to induce ballooning degeneration of hepatocytes and the presence of ghost cells on the liver parenchyma of treated rats. These lesions were diffuse with no restricted areas. These findings corroborated with those described by Shivakumar *et al.*[20], indicating marked degenerative changes associated with doxorubicin treatment. Kalender *et al.*[21] documented that the hepatotoxic potential of doxorubicin may be due to the formation of reactive oxygen species induced by its unstable metabolite doxorubicin semiquinone, thus interacting with the cells' macromolecules leading to cytological damage[22].

Acetaminophen, one of the most important drugs used for the treatment of mild to moderate pain has been found to have a dose and frequency-related hepatotoxicity accounting for the majority of cases of drug-induced acute liver failure[23]. The histopathological findings in the liver section of the APAP-treatment group in this study reflected hepatotoxicity mainly at the centrilobular regions of the hepatic lobules. This finding was following the report of James *et al.*[24], who documented that acetaminophen hepatotoxicity causes necrosis in the centrilobular areas of the liver tissues when taken in an overdose. More so, the periportal infiltration by polymorphonuclear cells, as observed in this study, has similarly been documented by Madkour and Abdel-Daim[25]. The well-established mechanism of cell damage of acetaminophen is considered to be mediated by its metabolic activation to a highly reactive toxic metabolite known as N-acetyl-P-Benzoquinoneimine (NAPQI). This was achieved via Cytochrome p450 (CYP) enzymes activity which is normally conjugated by glutathione released hepatocellular leading to depletion of GSH. Sener *et al.*[26] also documented that it could be a result of the generation of toxic oxygen species during the metabolism of acetaminophen to NAPQI leading to oxidative stress.

Upon treatment with the anti-tubercular (ATB) drugs, the lesions observed in the liver including cytoplasmic vacuolation and hepatocytes degeneration are also suggestive of marked hepatocellular damage. Both RMP and INH can establish liver injury when administered singly although RMP-mediated liver injury occurs much earlier than IND[27]. Co-therapy with RMP and INH work synergistically to enhance hepatocellular damage in a manner that is more deleterious than when they are administered as single treatments[28,29]. It is explained that RMP induces the major enzyme which is responsible for the metabolism of INH, thereby producing increased amounts of the toxic metabolites in the hepatic tissue[27]. Furthermore, pyrazinamide (PZM) was reported to cause more hepatotoxicity than RMP and INH[30], although the mechanism for PZM-induced toxicity is unknown[31]. It remains indisputable, however, that one of the important mechanisms of hepatotoxicity produced by ATB drugs is majorly due to induction by oxidative stress[32]. The evidence of vacuolated hepatocytes, in the present study, is typical of ATB drug-induced hepatotoxicity and this leads to a loss of the polyhedral structure of the hepatic cells[28].

Considering the histopathological findings on the kidney sections of treated rats, enlargement of the glomeruli was consistent with both DOX and APAP treatments while those in sections from ATB treatment revealed glomeruli lobulation and hypocellularity. The resultant effect of glomeruli enlargement is mostly an adhesion of the capillaries on the Bowman's capsule. This glomerular tuft adhesion was observed to be more severe with DOX treatment than with APAP treatment in this work. Tuft-to-capsule adhesion by DOX administration can relate to previously documented findings[33,34]. The APAP is scarcely reported to cause tuft-to-capsule adhesion but shrunken degenerating glomeruli have been reported after a 2 weeks daily dose of 2 g kg^{-1} [35]. Surprisingly, another study reported normal glomerular morphology after a cumulative dose treatment of up to 2 g kg^{-1} given in 2 weeks at 175 mg kg^{-1} daily intraperitoneally[36]. Perhaps the difference with the findings in this work may be the quantity administered daily, route of administration and/or maximum dose of APAP treatment achieved by the end of the study. On the other hand, the lobulated hypocellular nature of glomeruli observed upon ATB-treatment are evidence of nephrotoxic activity and this corroborates with the report of Muzika *et al.*[37], who observed lobulation of glomerular capillary loops and Prince *et al.*[38], who documented decreased cellularity of glomeruli of treated rats following a 21 and 28 days administration of INH and RIF, respectively. Since these effects were noticed in the present study with a shorter duration of treatment, it may be deduced that damage to the glomerulus upon INH and RIF treatment can be observed early within a few days of administration. The question concerning the effect of co-administration with

Pyrazinamide is yet to be addressed since the observed effects in the present study are similar to those obtained with just INH and RIF co-treatment[37]. Perhaps this suggests the reason why researchers choose to use INH and RIF to establish nephrotoxicity in animal models in search of natural products with nephroprotective effects[39-41]. However, it is worth noting that there is a paucity of scientific data on PYZ-induced nephrotoxicity but its addition to INH-RIF increases the risk of hepatotoxicity[42].

Another striking differentiating feature between the nephrotoxic activities of DOX, APAP and ATB in the present study is the effects on the tubules. Histoarchitectural changes including tubular dilation, degeneration, presence of eosinophilic casts within the lumen of the cortical tubules and sloughing of collecting ducts within the medulla were observed after the 7 days treatment with APAP. The ATB treatment caused more severe deleterious tubular changes as most of the tubules revealed marked dilation, shrunken epithelia, degeneration and necrosis. Previous reports on acute tubular degeneration and/or necrosis have often been implicated with APAP and ATB treatments[36,39,41,43] thus corroborating the findings in this work. Contrarily, the observed findings of the present study may not support the reports of Venkatesen and Deecaraman[44], which showed that the renal effects of acetaminophen were less commonly seen than the hepatic effects.

It should be noted that the non-involvement of the tubules with DOX treatment in the present work is not consistent with previous reports which documented tubular injury following adriamycin (doxorubicin) treatment for 16 weeks[33]. In their work, the loss of renal function observed was attributed to the extension of glomerular injury leading to the destruction of the tubule neck. Although the researchers could not proffer an explanation for the loss of tubules, they concluded that tubular injury associated with DOX is mostly secondary to glomerular damage and proteinuria or direct toxicity of the drug to the tubules[33]. Perhaps the shorter duration of DOX administration (7 days) in the present study may have accounted for the non-involvement of the tubules.

CONCLUSION

Data from this comparative study showed that the administration of doxorubicin, acetaminophen and a combination of first-line anti-tubercular drugs (rifampicin, isoniazid and pyrazinamide) showed distinct characteristic features at microscopical levels in the liver and kidney samples of albino rats.

SIGNIFICANCE STATEMENT

The present study discovered the different patterns of injuries induced by short-term administration of doxorubicin, acetaminophen and anti-tubercular drugs on the liver and kidney tissues of albino rats. This study will help and guide researchers on the pathological lesions they require at the microscopical level while using these drugs for a short duration to enable them to make proper adjustments on the dose of treatment or the toxicant of choice while investigating potent hepatoprotective and/or nephroprotective agents.

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