Original Research Article

Biochemical, Histopathological and Mutagenic Changes Following the Co-Administration of Antihelminthic and Antimalarial Drugs in Wistar Rats

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ABSTRACT

Aim
To determine the effects of antimalaria and antheminthic drugs combination in the incidence of histopathological alteration and biochemical modulations in liver and kidney of albino rats.
Place and duration of study
The study was undertaken at the Zoology Department University of Lagos Akoka Lagos Nigeria.
Methodology
A total of twenty (25) Male adult albino rats of 13-15 weeks old were divided into 5 groups of 5 rats each and daily oral administration of human therapeutic doses of praziquantel (PZQ 50mg/kg body weight) separate and in combination with ivermectin (IVM 0.4mg/kg body weight), albendazole (ALB 15mg/kg body weight) and Artemether-lumefanthrine (ACT 140mg/kg body weight) was administered with the group which serve as the control receiving 1ml distilled water. Toxic effects due to these treatments were investigated using histopathological, biochemical and mutagenic indices at day 8^{th} and 15^{th} of the study.
Result
Biochemical assessment revealed significant reduction in AST, ALT, ALP and potassium in the treatment group compared to the control. Increase in the level calcium, Albumin and bicarbonate were also observed in treatment groups. Histopathological assessment of the liver showed a general incidence of focal inflammation along the portal tract area, but did not show any differential severity across treatment groups except for single PZQ treatment group which were characterized by fatty infiltration. A general occurrence of mesangial damage and glomerula injury was observed in kidney tissues. Renal lesions were more severe in single PZQ +IVM treatment groups while mild lesions characterized renal tissue from PZQ+ACT treatment groups. Mutagenic effects as indicated by the high incidence of sperm head abnormalities was recorded across combination treatments especially in PZQ+ IVR and PZQ+ ACT groups.
Conclusion
Findings suggest that combination therapies are synergistic and could result in nephrotoxicity, antidiuretic effects, dehydration and mutagenicity at human therapeutic doses.

Keywords: Nephrotoxicity, Praziquantel, Combination-therapy, Human therapeutic doses, sperm head abnormalities

1.0 INTRODUCTION

The rise in global disease burden has seen an increased therapeutic use of drugs with unknown/poorly understood toxic potential [1]. Many of such implicated drugs include those with adaptable therapeutic applications, which often characterize interventions for public health issues like parasitic infections [2]. Recent reports indicate that parasitic and infectious diseases account for about 25% with a bulk of these incidences occurring in Africa, Southeast Asia and Eastern Mediterranean regions [2][3]. Some of the most documented incidences include high incidence of soil-transmitted helminthes infections among children [4] and maternal and infant mortality cases worldwide attributable to malaria annually particularly in Africa [5].

Aside fundamental factors like drug availability and costs, current therapeutic use and clinical discretion exercised during the application of antiparasitic drugs are largely guided by the increased incidence of drug-resistant parasites, and the characteristic narrow options of medications for parasitic infections [5][6]. Over time adaptive interventions for helminthic diseases and protozoan infections have included single-dose, safe, and relatively cheap drugs to drugs with a broad-spectrum activity, but with the incidence of drug-resistant pathogen species, elucidation and subsequent insight into the mechanisms underlying intrinsic and acquired drug-resistance has resulted in drug repurposing and development of rational combination therapies to overcome toxicity and resistance [7].

The therapeutic administration of drugs and combination therapies have however demonstrated potential for tissue injury or toxicity even when introduced within specified therapeutic ranges [8][1]. Such toxicity may result not only from direct toxicity of the primary compound but also from a reactive metabolite or from an immunologically-mediated response affecting particular cells or tissues [9] which in turn could result in pathological outcomes [10].Other studies have implicated the administration of drug combinations with an increased production of Reactive Oxygen Species (ROS) [11]. Post-drug intake effects in organs have been a key strategy for monitoring and determining drug-related toxicities [12].

It is against this background that this study investigated the role of antimalaria and

anthelminthic drug combinations in the incidence of histopathological alterations and biochemical modulations in liver and kidney of Albino rats and also observing possible mutagenic changes.

2.0 MATERIALS AND METHOD

2.1 Test animals

A total of twenty-five (25) male adult albino rats (*Rattusnorvegicus)* Wistar strain of 13-15 weeks old with an average weight of 180g±20 were used for the studies. The animals were purchased from an animal farm located in Ikorodu Lagos Nigeria and were maintained in the laboratory for 15 days with cross ventilation at controlled room temperature (27±2ºC) and relative humidity (40-60%) with a 12-hour light and dark cycle to acclimatize in the laboratory before the commencement of exposure period. All the rats were housed in conventional plastic cages. These standard cages were bedded with dry wood shavings, which were changed every 2 days to prevent maggotry. The animals were provided daily with fresh supply of standard feeds weighing 150g and water *ad libtum*.

2.2 Drug treatment and Sample preparations

Praziquantel (PZQ), Albendazole (ALB), Ivermectin (IVM) and Artemether-Lumefantrine (A-L) were used for study. The praziquantel tablet manufactured by BDH industries limited Mumbai india was purchase from a local pharmacy in Lagos Nigeria. IvermectinMectizan® a product of Merck& Co., Inc., Whitehouse station, New Jersey, USA was obtained from D-hub pharmacy Ikeja. Albendazole (Zentel) manufactured by SmithKline Beecham laboratories pharmaceuticals France and Artemether-Lumefantrine (Lonart Ds) manufactured by Bliss GVS pharmacy limited India was purchased from the University of Lagos community pharmacy. The drugs were grounded separately with mortar and pestle, weighed and measured at different concentration depending on the mean body weight of the experimental groups.

2.3 Experimental Design

Before exposure physical parameters such as laboratory temperature and humidity was determined. The human therapeutic dose for each drugs PZQ, IVM, ALB and ACT are 50mg/kg, 0.4mg/kg, 15mg/kg, and 140mg/kg body weight respectively. For the experiment there were 5 groups containing 5 rats (Table 1).

Table 1: Exposure group and treatments

S/N	GROUPS	MEAN WEIGHT OF RATS (g)	DRUG ADMINISTERED
1	Control	141.2	1ml distilled water
2	PZQ alone	182.4	Praz 9.12mg
3	PZQ + IVM	190	Praz 9.5mg +Ivr 0.08mg
4	PZQ+ ALB	166	Praz 8.3mg + Abz 2.49mg
5	PZQ + ACT	147.8	Praz7.39mg +ACT 20.7mg

2.4 Drug administration

The administration of drugs commenced 15 days after acclimatisation as described by Ismail *et al* [13] using oral route for 15 days for all groups except for group 5 in which ACT was administered at the last 3 days of exposure, after which they were sacrificed 24hrs after the last dose was administered based on the methodology by [14]. Animals were weighed after acclimatisation on the first day of exposure and the record served as the initial body weight (Day 0). The procedure was repeated on the 8^{th} day of exposure and before sacrificing at the expiration of the required time of exposure and value obtained served as the final body weight. The animals were observed daily for any clinical sign or behavioral changes.

2.5 Collection of blood and tissues

Blood specimen was collected in lithium heparin bottles and fluoride oxalate bottles. Liver, kidney and the cauda epididymis were excised. The cauda epididymis was used for

mutagenicity examination. The internal organs were placed in a plain bottle and Buoin's fluid added to preserve the specimen for histopathological examination.

2.6 Biochemical analysis

The method according to [15][16][17]was used to determine the biochemical parameters. Blood sample collected during heart excision of rats was used for quantitative determination of protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), urea, total bilirubin and creatinine, inorganic phosphate, cholesterol, fasting glucose, Na^{+}, K^{+}, Ca^{++} and Cl^{-} using standard kits.

2.7 Histological Preparations

Representative liver tissue of each group was excised, trimed of fat and other connective tissue and prepared for histological studies. The tissue samples were fixed using 10% normal saline for 24 hours and were later transferred into alcohol to remove excess water. Thin section (4-5m) were cut and stained with hematoxylin and eosin (H&E) stain. Thereafter the slides were examined under CX21 Olympus microscope of magnification of 40X objective and their photomicrograph taken with a Canon (Meville, NY) Power Shot G2 digital camera.

2.8 Mutagenicity Assay

Mutagenicity was determined from sperm head abnormalities. Four (4) male rats were sacrificed for each group by cervical dislocation after anesthetization. The caudaepidydimis excised from the male rat were placed in a Petri-dish containing 1ml of physiological saline and then minced and teased carefully well with fine scissors and forceps to release the spermatozoa. After gentle pipetting, the suspension is separated from the tissue fragments and a drop of 1% Eosin Y solution in the ratio (10: 1) was added to the suspension for 30 minutes. Air-dried smears were prepared on clean, grease-free glass slides using another clean slide angularly positioned at 45^{o} to spread the drop through the whole length of the slide. The slides were then coded, randomized and cytologically examined under a binocular

light microscopy with 400x magnification. Sixteen separate slides were prepared for each group for sperm examination. For each group, 2000 sperm cells were assessed for morphological aberration according to the criteria of [18]. The percentage abnormality of the sperm cells in the rats was calculated by using the mean value of the group.

$$\% \text{ abnormality} = \frac{\text{Total no of abnormal sperm cells}}{\text{Total no of sperm cells}} \times 100$$

2.9 Statistical Analysis

All data were expressed as mean ± standard deviation. One-way analysis of variance followed by Dunnett T_3 post hoc test was used for determining the statistical significance of the data. A probability level of less than 5% ($p<0.05$) was considered significant in all instances. All statistical tests were performed with SPSS 21 version package and originlab version 9.0.

3.0 RESULTS

3.1 Weight change across drug-treatment groups

Change in weight of experimental animals was assessed at 8^{th} and 15^{th} day intervals during the treatment period. Findings showed that exposure groups showed the highest weight change occurred in the drug-treatment groups particularly in single praziquantel exposure and Albendazole combinations. Both treatment groups showed higher significant weight difference at the beginning and end of the experiment when compared to control and Praz + ACT treatment group (Figure 1).

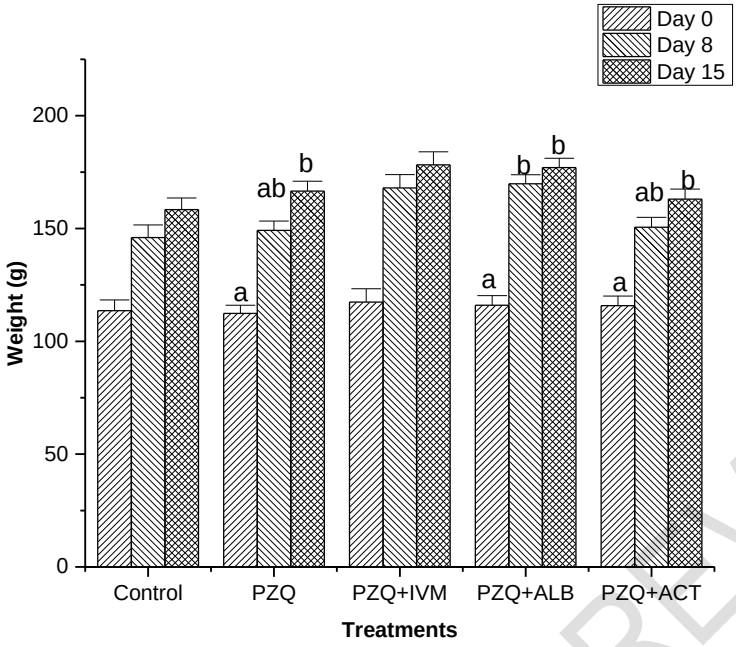

Figure 1: Weight change across control and drug treatment groups (bars within the same group with the same alphabet are not significantly different, where error bar=standard error)

3.2 Histopathology

Histopathology for liver on slides C, D, E, show focal inflammation with subtle features of hepatocyte loss. Appearance of these cells suggests focal loss which can be through apoptosis/necrosis (Figure 2).

For the kidney sample, Plate A which is the control showed subtle features of lobulation of the glomeruli. While Plates B and C i.e. PZQ and PZQ+IVM administered rats respectively showed significantly higher levels of severity compared to plates D and E (Figure 3).

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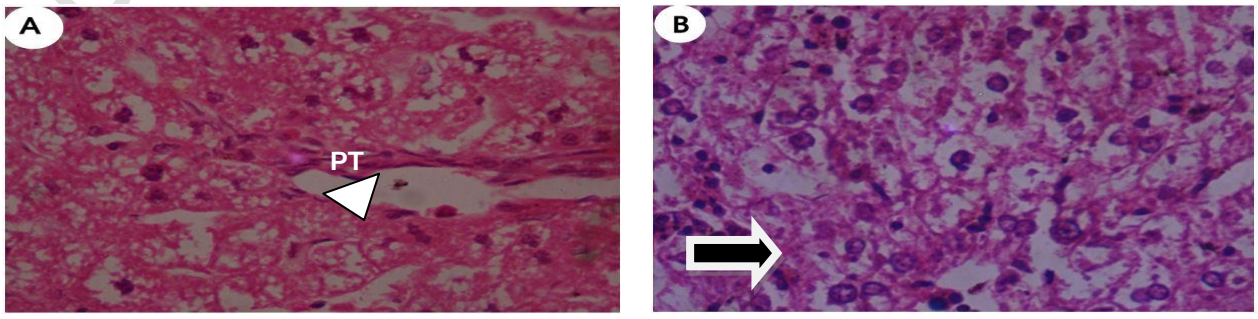

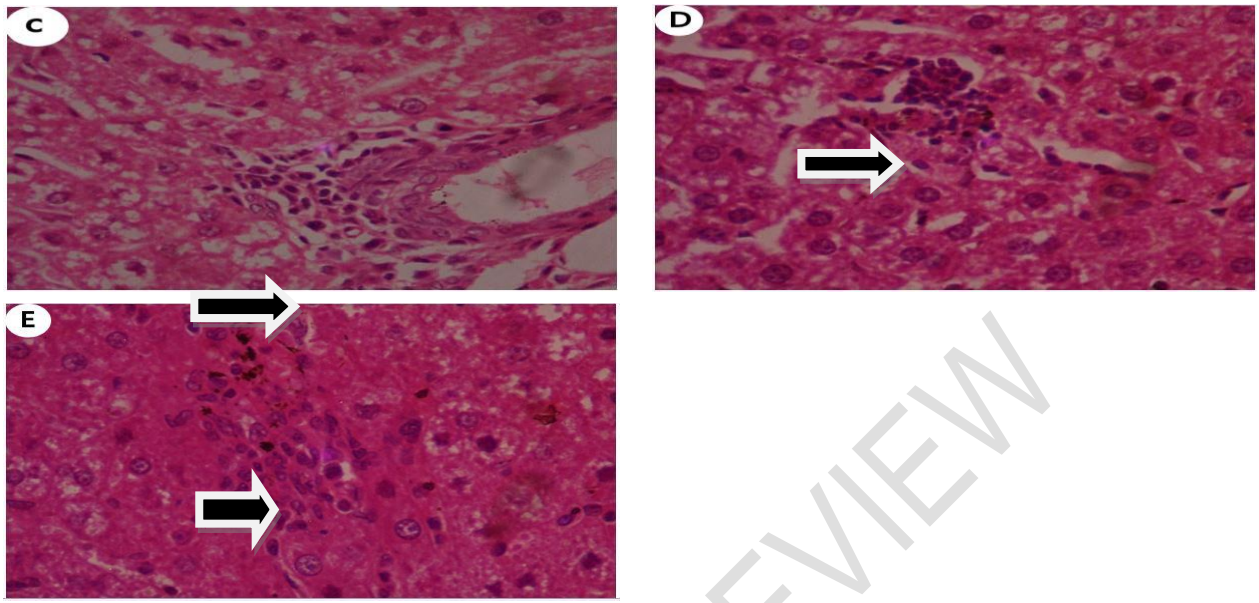

Figure 2: A: Histological section of liver tissue of control albino rats showing normal hepatocytes, bi-nucleated cells, cytoplasm and nucleus surrounded by a nuclear membrane and nucleolus around the portal tract area (PT) (arrow head) (Magnification X40) B: Histological sections of liver of albino rat administered PZQ showing fatty infiltrations (long arrow) C: Histological section of liver of albino rat administered PZQ+IVM showing focal inflammation (long arrow) around hepatic portal tract area with subtle features of hepatocyte loss. D: Histological sections of liver tissue of albino rat administered PZQ+ALB showing focal inflammation (long arrow) with subtle features of hepatocyte loss. E: Histological section of liver tissue of albino rat administered PZQ+ACT showing focal inflammation (short arrow) with subtle features of hepatocyte loss.

3.3 Biochemical Analysis

Liver enzyme profile across experimental groups showed that at least one treatment group have significantly lower levels of AST, ALT and ALP (Table 2). Also result of analysis showed that drug-treatment groups showed significantly higher levels of albumin compared to the control while creatinine was higher in serum of control animals. Other biomolecule variables such as glucose, urea and cholesterol did not differ significantly between drug-treatment groups and control (Table 2).

Electrolyte profile analysis depicted that ALB+IVM combination treatments showed significantly higher levels of sodium ion in serum compared to the control, while all treatment groups showed significantly lower levels of potassium ion compared to the control. All treatment groups showed significant elevated levels of calcium ion in serum compared to the

control while all treatment groups except the ACT combination treatment group showed significantly lower levels of phosphate ion in serum compared to the control. Bicarbonate ion levels were significantly elevated in treatment groups compared to the control while significant loss of chlorine ion in serum was recorded in the IVM drug-treatment group compared to the control.

Table 2:Analysis of biochemical variables in rats from control and PZQ, PZQ+IVM, PZQ+ALB and PZQ+ACT treatment groups.

Drugs exposure	AST (µ/L)	ALP (µ/L)	ALT (µ/L)	GLU (mmol/l)	UR (mmol/l)	ALB (mmol/l)	CRE (mg/d)	CHO (mmol/l)
Control (µ/L)	94.40 ± 27.07	44.64±10.59	32.00±14.93	5.32 ±0.83	5.10 ±3.09	37.46±2.8	60.60±7.3	2.10±0.4
PZQ (µ/L)	44.60 ± 15.13	33.14 ± 7.58	22.80 ±7.67	5.96 ±2.38	6.58 ±1.37	40.00±2.9	52.92±3.1	2.18±0.2
PZQ+IVM (µ/L)	48.50 ± 13.17	42.25 ±4.22	23.50 ±12.38	5.40 ±3.16	4.50 ±2.57	32.04±174	39.52±2236	1.82±1.6
PZQ+ALB (µ/L)	44.60 ±13.09	38.84 ±3.81	20.80 ±2.59	5.16 ±0.59	5.89 ±1.07	39.98±0.8	51.82±4.32	2.36±0.7
PZQ+ACT (µ/L)	57.40 ± 9.13	41.42 ±4.88	25.20 ±1.79	5.06 ±1.25	6.36 ±1.16	38.44±2.6	52.18±2.27	2.32±0.9

AST=Aspartate aminotransferase, ALP=Alanine phosphatase ALT=Alanine aminotransferase GLU=Glucose, UR=Urea, ALB=Albumin, CRE=Creatinine, CHO=Cholesterol

Table 3: Electrolyte variables in rats from control and PZQ, PZQ+IVM, PZQ+ALB and PZQ+ACT treatment groups.

Concentration of serum electrolytes	Control (µ/L)	PZQ (µ/L)	PZQ+IVM (µ/L)	PZQ+ALB (µ/L)	PZQ+ACT (µ/L)	Reference values
Na^+(mmol/l)	142.9 ±5.52	142.6±1.22	115.6±6.66	143.7 ±1.01	142.0 ± 2.35	144.33meq/l
K^+(mmol/l)	7.39 ±1.95	5.32 ± 0.62	4.73 ±2.67	5.75 ± 0.36	5.83 ± 0.58	5.26meq/l
Ca^{2+} (mmol/l)	1.68 ± 0.09	1.99 ± 0.22	1.69 ± 0.98	1.87 ± 0.18	1.88 ± 0.13	10.17mg/dl
PO_4^{2+}	1.42 ±0.13	1.07± 0.25	1.04 ±0.05	1.31± 0.34	1.24± 0.22	1.8-2.3
HCO_3(mmol/)	12.00 ±4.79	15.40±3.91	12.00 ±7.04	14.20 ± 2.59	14.00 ±4.64	18-30
Cl^{2+}	102.0 ± 2.83	100.4±1.82	82.00 ± 4.8	102.6 ± 2.70	103.6 ±1.52	103.75meq/l

Na^+= Sodium, K^+= Potassium,Ca^{2+}= Calcium, PO_4^{2+}= Phosphate, HCO_3= Bicarbonate
Cl^{2+}= Chloride

3.4 Sperm head abnormality assessment

Five different forms of sperm head abnormality were observed in the rat during the *in vivo* evaluation of the drugs. These include pin head (most prominent), no hook, hook at wrong angle, amorphous and bent sperm. The pin head sperm abnormality appeared predominantly in both the control and exposed group.

Table 4; Showing abnormal sperm cell recorded in experimental rats across treatment and control groups

GROUP	I.D	Normal	Amorphous	Bent	Wrong angle	No Hook	Pin head	Mean abnormal sperm cell	Mean no. of sperm cells	% Abnormal sperm
CONTROL	1	1670	30	45	-	-	-	68.25±49.14[a]	1751.8 ± 52.43[a]	3.8
	2	1708	30	7	28	-	38			
	3	1721	2	-	-	-	2			
	4	1635	-	-	-	-	60			
PZQ	1	1773	64	-	-	-		356.5±246.2[a]	1677.0±226.38[a]	21.25
	2	1384	44	-	40	-	250			
	3	680	176	395	30	-	65			
	4	1445	67	-	135	-	160			
PZQ+IVM	1	1281	209	123	123	-	238	710.8±182.3[b]	1561.8±607.32[a]	45.51
	2	689	210	175	-	-	270			
	3	235	35	154	20	-	20			
	4	1199	82	144	292	25	258			
PZQ+ALB	1	640	472	-	14	-	-	581.3±126.8[ab]	1397.8±254.52[a]	41.58
	2	1026	281	4	276	-	151			
	3	869	233	15	118	-	94			
	4	731	230	89	43	-	305			
PZQ+ACT	1	731	180	134	37	-	6	809.0±410.9[ab]	1676 ± 498.01[a]	48.26
	2	972	267	224	175	-	342			
	3	849	45	240	-	-	313			
	4	908	551	247	91	-	468			

3.5 DISCUSSION

A number of drugs with poorly understood scope of toxicity currently constitute drug options for public health interventions, particularly for parasitic diseases which have a high incidence among developing nations [5][19].As such a necessary step to avert drug-related biochemical disruption, pathological outcomes and mutagenic effects is to adequately examine and profile the toxic potential of drugs commonly used for public health interventions [1][20].

The biochemical modulations observed across drug-treatment groups represented in this study presents very interesting findings. The characteristic concurrent increase in albumin and calcium in both single PZQ drug treatment and PZQ+IVM treatment groups strongly highlight dehydration of animals in the both group. Dehydration has been implicated as a common cause of mild or transient hypercalcemia because when there is less fluid in the blood calcium concentrations rise [21]. The possibility of dehydration was also confirmed from the PCA where a negative correlation between PZQ, PZQ+IVM groups and serum phosphate was depicted. The negative correlation suggests hypophosphatemia which could also be diagnostic for dehydration. Also from the PCA, the positive relationship between albumin and Ca in these treatment groups could be explained on the basis that albumin binds calcium, thyroid hormones, fatty acids, and many drugs, keeping them in the blood circulation and preventing them from being filtered out by the kidneys [22]. The importance of albuminin the effectiveness and toxicity of therapeutic drugs and in drug interactions has been documented [22]. Furthermore, the negative correlation of these treatment groups with chloride (Cl^-) indicates decreased chloride levels in serum of these drug treatment groups. This decrease in serum chloride levels is diagnostic of tendencies towards hypochloremic alkalosis. Since this is an acute drug treatment study, could be described as acute hypochloremic alkalosis.

On the other hand, the strong negative correlation between the PZQ+ALB drug treatment group with potassium ion is suggestive of hypokalemic tendencies, while its positive association with sodium ion highlights hyponatremia which is also suggestive of dehydration. The combination of these two conditions highlights possibilities of metabolic alkalosis. Studies have shown that the kidneys compensate for loss of potassium by retaining sodium in the collecting ducts at the expense of hydrogen ions (sparing sodium/potassium pumps to prevent further loss of potassium), leading to metabolic alkalosis [23][24]. The strong positive correlation between the PZQ+ALB treatment group and bicarbonate levels confirms the possibilities of metabolic alkalosis [25]. Although this altered electrolyte levels may can be

attributed to the drug treatments, such patterns of electrolyte alterations may imply severe deleterious outcomes to patients with individual physiological risk factors e.g. advanced age, hypertension, gout and hyperuricaemia, diabetes mellitus, chronic renal failure and use of diuretics. Hypercalcaemia observed in single PZQ and combinations with IVM has been reported to enhance nephrotoxic drug injury by inducing pre-renal physiology [11]. Metabolic alkalosis which was also diagnosed in the treatment groups can result in alkaline urine which increases precipitations of drug crystals within the tubular lumen of the kidney [11][26]. In general, it was inferred that the single and combination PZQ treatment groups except PZQ+ACT demonstrated anti-diuretic symptoms and tendencies towards metabolic disruptions.

Although focal necrosis and inflammation of portal tract were common features across all PZQ drug combination, the absence of gradient or severity across treatment groups highlight one of the non-specific possibilities of histopathological assessment. Studies have noted that drug-related injury can mimic all the patterns observed in primary liver disease, making unequivocal histological diagnosis difficult or almost impossible in the majority of the case [9]. Findings from this study juxtaposed with relevant literature indicates that the PZQ combination treatment groups were likely to depict incidence of acute hepatitis. Ramachandra and Kakar [9] noted in their review of drug-induced liver disease that one of the hallmarks of acute hepatocellular injury are portal and parenchymal inflammation, hepatocellular injury and/or necrosis. Foci of inflammatory cells have been reported to occur spontaneously in livers of rodents in prechronic studies [27]. Other studies have also confirmed that inflammatory cell aggregates may be accompanied by evidence of hepatocellular necrosis [28][29].

The fatty infiltration (steatohepatitis or steatonecrosis) observed in liver tissues from the PZQ treatment demonstrates onset of liver degeneration. Drugs or their metabolites could inhibit esterification of fatty-acid within the hepatocyte resulting in hepatic vesicles engorged with

fatty acids [30]. Such drug-related incidences have been reported for alcohol i.e. alcoholic fatty disease [31] tetracycline [32] and Sodium valproate [33].

The more distinct pathology observed in kidney tissues across drug-treatment groups. This trend is expected because pharmacokinetic studies of PZQ reveal that in spite of the large absorption that occurs within the gastrointestinal tract (about 80%), only a relatively small amount enters systemic circulation due to extensive first-pass metabolisms. As a result, PZQ and its metabolites are mainly excreted renally within 24 h after a single oral dose, 70 to 80% is reportedly found in urine, but less than 0.1% as the unchanged drug [34][35]. This implies that PZQ will have more interaction with the kidney compared to the liver. Reports have shown that the role of the kidney as a primary eliminator of exogenous drugs and toxins makes it vulnerable to develop various forms of injury [20].

Furthermore, the realization that PZQ is metabolized through the cytochromeP450 pathway via CYP3A4 also highlights risks for the kidney. This is because CYP450 which constitutes part of the renal enzyme systems favours the formation of toxic metabolites and reactive oxygen species [36][35][37]. The presence of these by-products of metabolism tilts the balance in favour of oxidative stress, which outstrips natural antioxidants and increases renal injury via nucleic acid alkylation or oxidation, protein damage, lipid peroxidation and DNA strand breaks [36][38].

The mild mesangial damage in single PZQ drug treatment group compared to the severe mesangial damage in PZQ+IVM and PZQ+ALB treatment groups, suggests that ivermectin and albendazole could enhance renal toxicity. Incidence of proximal cell tubular toxicity is indicative of drug-induced nephrotoxic effects e.g. phospholipid damage, increased intracellular calcium concentrations. Other effects include osmolar effects with loss of normal cell contact and tubular occlusion [37][39].

The mutagenicity test as indicated by the occurrence of sperm head abnormality, recorded high incidence of abnormality in all drug treatment groups. The higher incidence of abnormality in PZQ+ IVM and PZQ+ACT were statistically significant ($P<0.05$). The predominance of pinhead sperms over all other varying types of sperm head abnormality in the treated groups is consistent with reports on PZQ administered to albino mice for a period of 5-8 weeks [40]. The non-significant difference in incidence of sperm head abnormalities between the control group and PZQ treatment group confirms early reports on the non-mutagenic potential of PZQ treatments in humans [41][42]. Considering the non-mutagenic effects of PZQ demonstrated from this study and the non-mutagenic potential of IVM earlier reported [43], mutagenic effects of combination therapies may be attributed to synergistic interaction of the drugs.

4.0 CONCLUSION

Identifying drug-related risks and drug-induced injury is the key to reducing risk of damage to vital organs as liver and kidney. Findings from this study depict that single praziquantel administration and combinations with Ivermectin and albendazole at human therapeutic doses portends risks of liver inflammation, while combination treatments are most likely to induce metabolic disruptions, antidiuretic effects and likelihoods of weight gain due to dehydration. Combination treatments are also likely to induce mutagenic effects as indicated by higher incidence of sperm head abnormalities.

Since drug-related risk factors are one of many factors that influence liver and kidney toxicity, more extensive profiling of common drugs options for public health interventions is recommended. This will inform clinical decisions that could increase the risk factors and deleterious outcomes of patients.

COMPETING INTERESTS

There is no competing interest among authors.

CONSENT

Not applicable.

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