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Artemether-Lumefantrine treatment combined with albendazole and ivermectin induced genotoxicity and hepatotoxicity through oxidative stress in Wistar rats

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## a b s t r a c t

Mass drug administration against malaria and parasitic worm co-infections is capable of increasing health risk. This study investigated the hepatotoxicity, genotoxicity and oxidative stress induced by combinations of Arthemether-Lumefantrine (A-L) with Albendazole (ABZ) and Ivermectin (IVR) treatments in rats. 65 rats equally distributed into 13 groups were orally gavaged human therapeutic doses (×1.0), half of the doses (×0.5) and twice the doses (×2.0) of these drugs per body weights. Blood, liver and bone marrow cells were analyzed for serum biochemistry, histopathology and micronucleated polychromatic erythrocytes (MNPCE) respectively. Treated rats showed clinical signs of toxicity. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bili- rubin and malondialdehyde (MDA) significantly increase with concomitant decrease in superoxide dismutase (SOD) and catalase (CAT) in the serum. Liver histology revealed

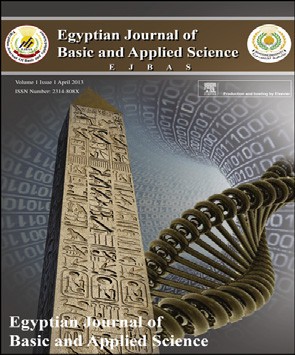
single cell hepatocellular necrosis and kupffer cell hyperplasia, multiple foci vacuolar changes in the hepatocytes, thinning of hepatic cord and congestion of the sinusoids by inflammatory cells. Also, frequency of MNPCE significantly increased in the treated rats.

The findings revealed that combine treatment of A-L with ABZ and IVR mostly at ×2.0

and ×1.0 induced liver dysfunctions and somatic mutations through oxidative stress in rats. These suggest health risk in wildlife and human populations during treatments with these drug combinations.

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1. Introduction

Parasites infect more than 200 million people worldwide. Chronic infestations may elicit inflammation, lead to cancer formation and deaths [[1]](#_bookmark10). Infections from parasitic worms and protozoa are the major cause of human and wildlife morbidity and mortality. In tropical and sub-tropical regions, *Plasmodium* species, the parasitic protozoan responsible for human and animal malaria, is the leading cause of morbidity and mor- tality with children highly vulnerable, accounting for about 1e2 million deaths annually [[2]](#_bookmark11). Similarly, schistosomiasis, lymphatic filariasis, ascariasis, enterobiasis and onchocerci- asis caused by helminthes and nematodes, are common health issues in most tropical and sub-tropical countries [[3]](#_bookmark12). In endemic situations, these protozoan and worms coexist in human and animals to cause severe infestation and death [[4]](#_bookmark13). In this situation, mass drug administration is usually the recommended treatment strategy for effective parasite clearance [[5]](#_bookmark14).

Coartem®, an artemisinin combination therapy containing

Artemether-Lumefantrine (A-L), is the leading and first line drug recommended for the treatment of acute and compli- cated malarial fever in patients with minimum 5 kg body weight [[6](#_bookmark15)e[8]](#_bookmark15). Clinical trials showed that Coartem® is effective, safe and has good tolerability against multi-drug resistant *Plasmodium falciparum* [[9,10]](#_bookmark18). It produces about 10,000 folds decrease in parasite biomass per asexual cycle [[11]](#_bookmark19). Albenda- zole (ABZ) and Ivermectin (IVR) are effective anti-parasitic agents usually recommended for the treatment of human and veterinary parasitic worm infections [[12](#_bookmark20)e[14]](#_bookmark20). Co- administration of these drugs with A-L during helminthic, nematode and malarial co-infection or resistance has shown to be effective. Horton et al. [[15]](#_bookmark22) reported that single dose of IVR and ABZ combined therapy against lymphatic filariasis produced better treatment efficacy than the individual drug effects. Mohammed et al. [[16]](#_bookmark23) also reported that co- administration of praziquantel and ABZ was effective against schistosomiasis and soil-transmitted helminthiasis morbidity. A-L a fixed-dose combination tablet of 20 mg artemether and 120 mg lumefantrine in the ratio 1:6 is effec- tive against complicated *P. falciparum* malaria [[8,17]](#_bookmark16). These reports are in support of the efficacy of mass drug adminis- tration against co-infections caused by parasitic protozoa and worms.

Considering that drugs are synthetic chemicals, they are

capable of producing both beneficial and harmful effects. It is important to evaluate the possible deleterious effects of administering mass drugs during protozoa and worm co- infections to avert the probable health effects. This is neces- sary considering that the harmful drug effects have been linked to liver damage, bone marrow toxicity, carcinogenesis and fetal developmental anomalies [[18,19]](#_bookmark24). It is plausible that combine drug administration against malaria and parasitic worm co-infection may elicit toxic effects in the host due to drugedrug interactions [[20]](#_bookmark26). *In vivo* and *in vitro* studies of in- dividual drugs; A-L, ABZ and IVR induced various toxicities in biological systems. Artemether treatment induced necrosis in gastric cancer cell line (PG100), and necrosis and apoptosis in human lymphocytes [[24]](#_bookmark28). ABZ induced cytotoxicity in liver

cells of treated rats via elevation of liver function enzymes and alterations in oxidative stress enzymes during sub- chronic exposure [[25]](#_bookmark29). IVR similarly induce cytotoxicity and genotoxicity in treated mice and Chinese hamster ovary cells [[20,26]](#_bookmark26). These reports showed that A-L, ABZ and IVR are potentially harmful to biological systems. They may be considered effective and safe at therapeutic doses, but an overdose may result into severe tissue injury, organ failure and death [[27]](#_bookmark30).

Liver, the most sensitive predictor of chemical induced toxicity due to its involvement in metabolism, detoxification and storage of drugs and their metabolites, is an important target organ for drug induced injury in mammals [[25,28,29]](#_bookmark29). Also, bone marrow the site of blood cell proliferation may be subject to drug induced toxicity during acute and chronic exposure [[25,26]](#_bookmark29). Considering that Artemether and Lumefan- trine (A-L), Ivermectin (IVR) and Albendazole (ABZ) may be ignorantly abused following the mass drug administration strategy in the control of malaria and parasitic worm co- infection. It is important to understand the possible mecha- nism of liver dysfunctions and bone marrow cell toxicity (genotoxicity) of combine treatments of A-L, IVR and ABZ in mammalian systems is linked to reactive oxygen species formation.

This study investigated the hepatotoxicity, genotoxicity

(using micronucleus assay) and alterations in serum antioxi- dant enzymes (catalase and superoxide dismutase) and lipid peroxidation as possible mechanisms of co-administrations of A-L, ABZ and IVR induced toxicity in Wistar rats.

1. Materials and methods

### *Chemicals*

Artemisinin-based combination therapy containing Arte- mether and Lumefantrine (A-L); Coartem® (Norvatis Pharma- ceuticals Corporation Suffern, New York, USA), Ivermectin (IVR); Mectizan® (Merck & Co., Inc., Whitehouse station, New Jersey, USA) and Albendazole (ALB); Expezol® (Swiss Pharma Nigeria Limited, Lagos), Cyclophosphamide monohydrate (Endoxan™ Mfg Lic. No. 186. Frankfurt am Main, Germany), positive control, Fetal calf serum (Sigma St Louis, MO, USA), Giemsa and May-Gru¨ nwald stains (Merck, Germany) used for this study were of analytical grades.

### *Animals*

Sixty five (65) male Wistar rats (between 7 and 8 weeks old) obtained from the animal unit, College of Medicine, University of Ibadan, Nigeria were used for the study. They were accli- matized for 14 days until they were 134.0 ± 2.0 g (mean ± SD) body weight. They were maintained in laboratory conditions of 12 h dark and light cycle, temperature of 27 ± 8 ◦C, relative

humidity of 69 ± 15% and had access to clean drinking water

and standard rodent chow (Ladokun feed Nigeria®) *ad libitum*. Guide for care and use of Laboratory Animals published by US National Institutes of Health (NIH Publication No. 85e23, revised in 1996), and approved by the ethical committee,

112 [e gypti an j o ur nal o f b a sic and a p p l i ed sci e n c e s 2 ( 201 5 ) 110](http://dx.doi.org/10.1016/j.ejbas.2015.03.001) e[119](http://dx.doi.org/10.1016/j.ejbas.2015.03.001)

University of Lagos for the use of animals in experimental studies was carefully adhered.

### *Experimental design and drug administration*

5 rats per group were randomly distributed into 13 experi- mental groups with cyclophosphamide (CYP, 20 mg/kg/bwt) and distilled water (vehicular solvent) used as positive and negative controls respectively. Human therapeutic doses for the three drugs (×1.0), sub-curative (half of the dose; ×0.5) and twice the doses (×2.0) were administered to the rats via oral gavaging according to their body weights (mg/kg). The experimental groups are A (negative control), B [human

therapeutic dose for Artemether-Lumefantrine, 20/120 mg/kg bwt (×1.0 AL)] [[7,10]](#_bookmark17), C [sub-curative human therapeutic dose for Artemether-Lumefantrine, 10/60 mg/kg bwt (×0.5 AL)], D [twice the human therapeutic dose for Artemether- Lumefantrine, 40/240 mg/kg bwt (×2.0 AL)], E [human thera- peutic doses for Artemether-Lumefantrine, 20/120 mg/bwt and Albendazole, 400 mg/kg bwt (×1.0 AL + ABZ)] [[39]](#_bookmark39), F [sub- curative human therapeutic doses for Artemether-

Lumefantrine, 10/60 mg/bwt and Albendazole, 200 mg/kg; (×0.5 AL + ABZ)], G [(twice of the therapeutic doses Artemether-Lumefantrine, 40/240 mg/bwt and Albendazole, 800 mg/kg (×2.0 AL + ABZ)], H [human therapeutic doses for Artemether-Lumefantrine, 20/120 mg/bwt and Ivermectin, 3 mg/kg (×1.0 AL + AVR)] [[13,41]](#_bookmark21), I [sub-curative therapeutic

|  |  |  |
| --- | --- | --- |
| Table 1 e Experimental animal grouping and drug dosage and administration. | | |
| Experimental groups | Drug doses (mg/kg bwt) | Exposure durations |
| A B C D E F G H I J K L  M | Distilled water | 0.5 ml for 3 consecutive |
|  | days |
| 20/120 AL | 0.5 ml for 3 consecutive |
|  | days |
| 10/60 AL | 0.5 ml for 3 consecutive |
|  | days |
| 40/240 AL | 0.5 ml for 3 consecutive |
|  | days |
| 20/120 AL + 400 | 0.5 ml AL + 0.5 ml ABZ for |
| ABZ | 3 days |
| 10/60 AL + 200 | 0.5 ml AL + 0.5 ml ABZ for |
| ABZ | 3 days |
| 40/240 AL + 800 | 0.5 ml AL + 0.5 ml ABZ for |
| ABZ | 3 days |
| 20/120 AL + 3 IVR | 0.5 ml AL + 0.5 ml IVR for |
|  | 3 days |
| 10/60 AL + 1.5 IVR | 0.5 ml AL + 0.5 ml IVR for |
|  | 3 days |
| 40/240 AL + 6 IVR | 0.5 ml AL + 0.5 ml IVR for |
|  | 3 days |
| 20/120 AL + 400 | 0.5 ml AL for 3days + 0.5 ml |
| ABZ | ABZ only on the 3rd day |
| 20/120 AL + 3 IVR | 0.5 ml AL for 3days + 0.5 ml |
|  | ABZ only on the 3rd day |
| 40 CYP | 0.5 ml for 3 consecutive days |
| AL = Artemether-Lumefantrine (Artemisinin-based combination therapy; Coartem®); IVR = Ivermectin; ABZ = Albendazole; CYP = Cyclophosphamide (positive control). Drugs were consti- tuted using distilled water (vehicular solvent; negative control). | | |

doses for Artemether-Lumefantrine, 10/60 mg/bwt and Iver- mectin, 1.5 mg/kg (×0.5 AL + IVR)], J [twice of therapeutic doses for Artemether-Lumefantrine, 40/240 mg/bwt and Ivermectin, 6 mg/kg (×2.0 AL + IVR)], K [therapeutic doses for

Artemether-Lumefantrine, 20/120 mg/bwt and single admin- istration of Albendazole, 400 mg/kg], L [therapeutic dose for Artemether-Lumefantrine, 20/120 mg/bwt and single admin- istration of Ivermectin, 3 mg/kg] and M [Cyclophosphamide, (CYP) 20 mg/kg]. The drugs were administered to the various groups for three consecutive days. Similar treatment was concurrently given to the negative control group, A (distilled water; vehicular solvent). [Table 1](#_bookmark3) presents the summary of the experimental design for drug administration to the various groups.

### *Clinical signs of toxicity and mortality*

Rats in each treatment group were observed twice daily (before and after exposure) for signs of clinical toxicity in the appearances of their skin and fur, eyes and mucous mem- brane, behavioral pattern, morbidity and mortality.

### *Serum biochemical analysis*

At the end of exposure periods, rats were fasted overnight and blood collected from the orbital plexus using heparinized 70 ml micro-hematocrit capillary tubes into lithium coated serum separator tubes. The clotted blood was centrifuged at 3000 g for 10 min to separate the serum (supernatant) and

stored at —70 ◦C prior to biochemical analysis. Serum

biochemical markers of oxidative stress were measured ac- cording to standard protocols: Catalase (CAT; EC 1.11.1.6) ac- tivity was measured according to the method of Aebi [[30]](#_bookmark32), superoxide dismutase (SOD; EC 1.15.1.1) activity was measured in accordance with Magwere et al. [[31]](#_bookmark33) method, while lipid peroxidation was measured as malondialdehyde (MDA) concentrations in accordance with Nichaus and Samuelson [[32]](#_bookmark34) method. Protein concentration was measured according to the methods of Lowry et al. [[33]](#_bookmark35). Serum liver function test markers; transaminases were measured ac- cording to Reitman and Frankel [[34]](#_bookmark36) and total bilirubin ac- cording to Treitz [[35]](#_bookmark37) using Randox Laboratory (UK) diagnostic kits. The absorbances for all the reactions were measured spectrophotometrically using HAICE®, DR 3000 (Germany).

### *Mammalian bone marrow micronucleus assay*

Bone marrow micronucleus test was conducted according to Schmid [[36]](#_bookmark38). Femoral bones from treated and control rats were surgically removed and the bone marrow flushed into eppendof tubes using 0.5 ml of Fetal Bovine Serum (FBS). The cells were centrifuged at 2000 rpm for 5 min and smear made on pre-cleaned grease free slides. Prepared slides were air dried and stained with May-Grunwald and Giemsa stains. They were coded and examined under an Olympus light mi-

croscope at 1000× magnification. 2000 cells per rat were

scored for micronucleated polychromatic erythrocytes (MNPCE).

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### *Histopathological analysis*

Slices of the right lobe of the liver tissue from drug treated and control rats were fixed in 10% neutral buffered formalin. The tissues were dehydrated in ascending order of ethyl alcohol-water concentrations, cleared in xylene and sequentially embedded in paraffin wax blocks using rotary microtome. Tissue sections of 3e5 mm thick were cut and prepared on clean slides for Hematoxylin-Eosin (H-E) staining before mounting in neutral DPX medium. Prepared

slides were examined at 400× magnification by trained

pathologist.

### *Statistical analysis*

All statistical analyses were conducted with Graphpad prism 5.0® computer programs. One-way ANOVA was used to determine the differences (p < 0.05) among the various treated groups and control. Difference between each treat- ment group and the negative control was determined using comparison procedure of the Dunnett multiple post-hoc test (DMPT). Significance was considered at p < 0.05; p < 0.01 and p < 0.001.

1. Results

### *Clinical signs of toxicity and mortality*

During the exposure periods, two animals died, one each in groups E and G. The survivors in groups D, E, G, H, J, K and L exhibited sluggishness and weak movement (signs of reduced activities), diarrheae, ungroomed fur and labored breathing movement. They also exhibited reduction in their feeding habit. A rat in the H group developed abscess in the thigh re- gion of the right leg ([Fig. 1](#_bookmark4)). These signs of toxicity were rarely observed in rats from groups B, C, F and I and none was observed in the negative control group. There was no signifi- cant change in the body weight gain and liver weight gain between treatment groups and the negative control (data not shown).

### *Biochemical indicators of hepatic function, lipid* peroxidation and oxidative stress

[Fig. 2](#_bookmark5)(aec) presents the results of the serum hepatic function tests. The activities of ALT, AST and total bilirubin concen- tration significantly (p < 0.001) increased in rats exposed to the drug combinations. Drug exposure to rats in groups D, E, G, H, J, K and L resulted in significant increase in ALT activity by 37.69, 38.52, 78.13, 58.72, 94.65, 50.66 and 71.94% folds

respectively as well as AST activity by 26.05, 34.74, 98.51, 67.29, 127.77, 66.42 and 81.41% folds respectively compared to the negative control. [Table 2](#_bookmark6) shows the results of the antioxidant enzyme activities and lipid peroxidation. SOD and CAT ac- tivities significantly (p < 0.05) decreased in the drug adminis-

tered groups compared to the negative control, with the

reduction dependent on drug treatment groups. The per- centage decrease for SOD activities in rats from E, G, H, L, K, and J groups were significantly different from the negative

control by 33.33, 40.33, 21.28, 18.41, 15.08 and 29.11% folds respectively. While the percentage decrease for CAT activity in rats from E, G, H, L, K and M groups are 31.82, 47.98, 22.22, 7.07, 17.68 and 31.31% folds respectively. Reduction in the activities of these enzymes is accompanied with concomitant significant (p < 0.05) increase in MDA concentration with

34.78, 73.46, 2.13, 11.0, 14.11 and 48.36% folds increase for rats

in the treatment groups: E, G, H, L, K and J respectively compared to the negative control. The results of the biochemical alterations showed positive correlations accord- ing to the drug administration ([Fig. 2](#_bookmark5)aec and [Table 2](#_bookmark6)).

### *Micronucleus analysis*

[Fig. 3](#_bookmark7) shows the genotoxicity data. There is significant in- crease (p < 0.05) in the frequencies of micronucleated poly- chromatic erythrocytes. The treatment groups: E, G, H, J, K and L were significantly higher than the negative control by 6.78, 8.12, 1.81, 3.11, 5.49 and 2.97 folds respectively. [Fig. 4](#_bookmark8) shows representative polychromatic erythrocyte (PCE) and micro- nucleated PCE scored for the genotoxicity assessment of the drugs.

### *Histopathological assessment of the liver*

[Fig. 4](#_bookmark8)aef presents the histological sections of the liver from the treated rats and the negative control. Sections of the liver from the negative control rats showed apparently normal hexagonal or pentagonal lobule of the hepatocytes. These cells are regular and contain a large spheroidal nu- cleus ([Fig. 4](#_bookmark8)a). Histology of the liver tissues from combined drug treated rats showed some distorted architectural structures from the negative control. These alterations are single cell hepatocellular necrosis and kupffer cell hyper- plasia, which were common among most treated rats in the following groups; D, E, G, H, J, K, L and the positive control. Also multiple foci vacuolar changes in the hepatocytes, thinning of hepatic cord and congestion of the sinusoids by inflammatory cells were observed in these treated groups ([Fig. 5](#_bookmark9)bef).

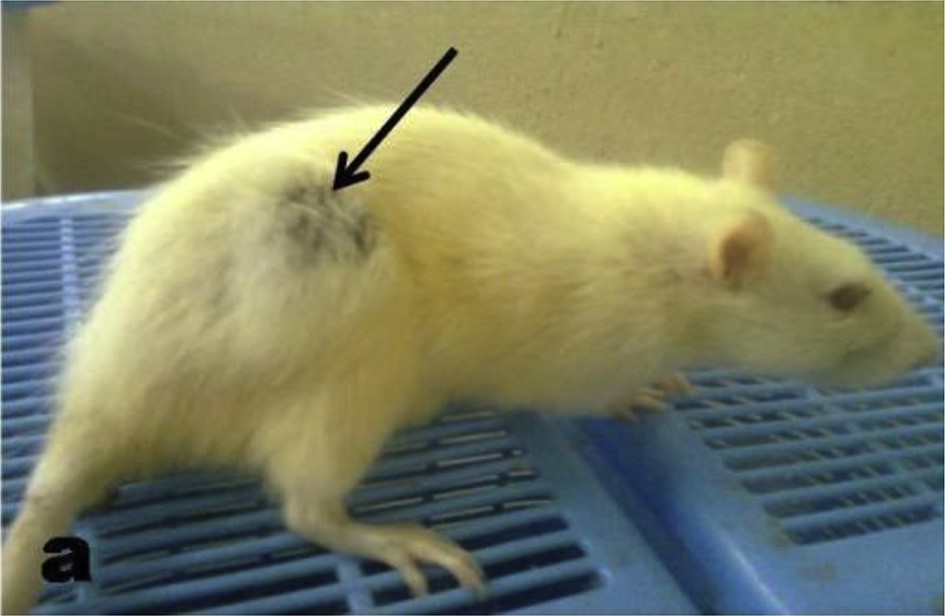


Fig. 1 e Abscess on the thigh region of the right leg of rat exposed to Artemether/Lumefantrine (20/120 mg/bwt) and Ivermetin (3 mg/kg), sign of clinical toxicity to drug exposure.

114 [e gypti an j o ur nal o f b a sic and a p p l i ed sci e n c e s 2 ( 201 5 ) 110](http://dx.doi.org/10.1016/j.ejbas.2015.03.001) e[119](http://dx.doi.org/10.1016/j.ejbas.2015.03.001)



# a



c

c

b

b

a

a

a

a

\* \*

\*

\*

p < 0.0001; r2 = 0.85

80

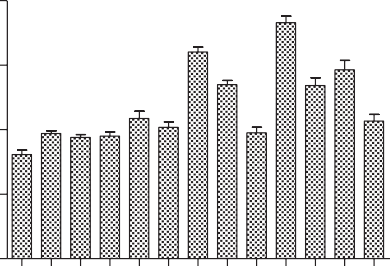
60

Serum ALT (IU/L)

40

**b** 200

150



c

c

b

b

b

a

a

a

\* \*

\*

\*

p < 0.0001; r2 = 0.91

Serum AST (IU/L)

100

20

0

A B C D E F G H I J K L M

Groups of experimental treatment

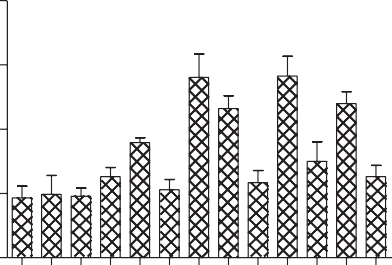
50

0

A B C D E F G H I J K L M

Groups of experimental treatment

0.8



c c

b b

a

a

\*

\*

\*

\*

\*

\*

p < 0.0001; r2 = 0.70

Serum Total Bilirubin (mg/dL)

**c**

0.6

0.4

0.2

0.0

A B C D E F G H I J K L M

Groups of experimental treatment

Fig. 2 e a. Effects of drug exposure on serum ALT activity of rat. End point represents mean ± SE for 5 rats. Values are significantly different; ap < 0.05; bp < 0.01; cp < 0.001 and \*p > 0.05 compared to negative control. b. Effects of drug exposure on serum AST activity of rat. End point represents mean ± SE for 5 rats. Values are significantly different; ap < 0.05; bp < 0.01; cp < 0.001 and \*p > 0.05 compared to negative control. c. Effects of drug exposure on total bilirubin concentration of rat. End point represents mean ± SE for 5 rats. Values are significantly different; ap < 0.05; bp < 0.01; cp < 0.001 and \*p > 0.05 compared to negative control.



1. Discussion

Mass drug administration strategy against protozoa and worm co-infections [[8]](#_bookmark16) is capable of increasing human expo- sure to multiple drug combinations. Mostly in developing countries where drugs are readily purchased from unregis- tered patent medicinal stores and hawkers without physician

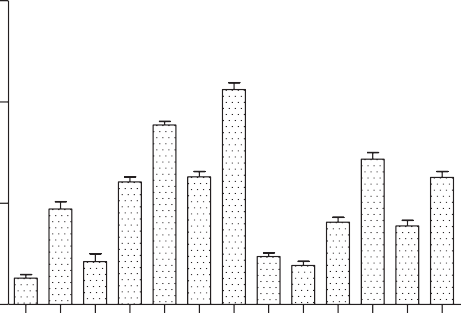
prescriptions. Free access to drugs, coupled with wrong application due to ignorance of drug toxicity may endanger human and animal health. Ivermectin, Albendazole and Artemether-Lumefantrine used in this study were selected based on their overwhelmingly and extensively use as broad- spectrum anti-nematode, anti-helminthic and antimalarial drugs respectively [[5,7,13,14,37]](#_bookmark14). Also individual toxic effects



|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Table 2 e Effects of drug combinations on serum CAT, SOD and MDA in treated and control rats. | | | | | | |
| Treatment | SOD | (95% CI) | CAT | (95% CI) | MDA | (95% CI) |
| A | 31.29 ± 2.43 | (27.32e35.26) | 1.98 ± 0.02 | (1.36e2.60) | 28.64 ± 1.16 | (25.42e31.86) |
| B | 29.73 ± 1.68\* | (27.49e31.98) | 1.86 ± 0.13\* | (1.22e2.49) | 32.72 ± 2.82\* | (27.66e37.77) |
| C | 29.72 ± 3.49\* | (25.57e33.87) | 1.94 ± 0.09\* | (1.41e2.48) | 29.62 ± 1.66\* | (25.01e34.23) |
| D | 23.63 ± 3.32b | (19.96e27.30) | 1.75 ± 0.17\* | (1.28e2.22) | 41.80 ± 0.96c | (36.37e35.88) |
| E | 22.86 ± 0.93c | (18.18e23.53) | 1.35 ± 0.21a | (1.04e1.66) | 38.60 ± 0.93b | (33.24e43.95) |
| F | 26.56 ± 0.95a | (21.16e31.97) | 1.66 ± 0.01a | (1.36e1.95) | 40.41 ± 1.49c | (33.50e47.31) |
| G | 20.65 ± 2.02c | (17.81e23.48) | 1.03 ± 0.08b | (0.26e1.80) | 49.68 ± 2.00c | (44.14e55.22) |
| H | 24.63 ± 1.45a | (23.59e31.67) | 1.54 ± 0.16a | (1.09e1.99) | 29.25 ± 3.08\* | (26.26e32.24) |
| I | 30.43 ± 0.68\* | (25.76e35.10) | 1.75 ± 0.26\* | (1.30e2.20) | 30.67 ± 1.13\* | (24.75e36.59) |
| J | 22.18 ± 1.61b | (17.75e26.88) | 1.36 ± 0.01a | (1.04e1.68) | 42.49 ± 0.38c | (35.88e49.10) |
| K | 26.57 ± 1.71a | (21.77e31.28) | 1.63 ± 0.20a | (1.07e2.19) | 32.68 ± 2.74a | (27.86e33.24) |
| L | 25.53 ± 1.16a | (24.31e30.75) | 1.84 ± 0.03\* | (1.47e2.21) | 31.79 ± 1.53\* | (27.54e36.04) |
| M | 25.48 ± 2.36a | (21.70e29.25) | 1.42 ± 0.13a | (1.06e1.79) | 35.72 ± 3.03a | (30.09e41.34) |
| End point represents mean ± SE for 5 rats. Values are significantly different; ap < 0.05; bp < 0.01; cp < 0.001 and \*p > 0.05 compared to negative control. SOD (superoxide dismutase; U/mg protein), CAT (catalase; mmol/mg protein), MDA (malondialdehyde; nmol/ml), (95% confidential interval). | | | | | | |

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15



c

c

b

b

b

b

a

a

a

\*

\*

\*

p < 0.0001; F= 107.4; r2= 0.96

MN PCE/2000 cells

10

5

0

A B C D E F G H I J K L M

Groups of experimental treatment

Fig. 3 e Frequency of micronucleated polychromatic erythrocytes in bone marrow cells of rats exposed to drugs and controls. End point represents mean ± SE for 5 rats. Values are significantly different; ap < 0.05; bp < 0.01;

cp < 0.001 and \*p > 0.05 compared to negative control.



of these drugs have been observed in mammalian systems [[21](#_bookmark27)e[23,25,27,38,39]](#_bookmark27), but there is dearth of information on the toxic effects of their binary combinations. This study corrob-

orated oxidative stress induced by binary combinations of AL, AL + ABZ and AL + IVR with structural and biochemical al- terations in the liver, and micronuclei polychromatic eryth- rocyte formation in the bone marrow cells of rats.

Clinical signs of toxicity observed in the treated rats in groups D, E, G, H, J, K and L suggest systemic toxicity of the drug combinations in the treated rats. Anorexia (loss of appetite) is a common symptom of liver injury attributable to drug exposure and is consistent with reduced activities; sluggishness and weakness [[40]](#_bookmark40). Labored breathing, diarrheae, ungroomed hair and hair loss observed in the treated rats are common symptoms of hepatotoxicity and immune systems dysfunction due to toxicosis from overdose of the adminis- tered drugs and their pharmacokinetics [[41,42]](#_bookmark41). Mortality

recorded at the ×1.0 AL + ABZ (group E) and ×2.0 AL + ABZ

(group G) treatment may be associated with overdose which caused acute toxicity. Abscess observed in a rat from group H (×1.0 AL + IVR) may be associated with cellular inflammatory actions induced by the drug combination. This assertion lends

credence to the observed necrotic cells in the liver of the ×1.0 AL + IVR treated rats. Moreover, high dose administration of

Ivermectin induced neutrophilic activation and eosinophilic bursting of the human peripheral blood [[43,44]](#_bookmark42) further sup- ported the cellular inflammatory actions of the drugs. Generally inflammatory cells; macrophages, neutrophils and lymphocytes function by destroying invading microorgan- isms, removal of necrotic cells (accidental cell death) and cellular debris caused by drugs and other xenobiotics during phagocytosis [[45,46]](#_bookmark43). This process is usually associated with intracellular ROS formation via autoxidation [[45]](#_bookmark43), which is in support of Hyslop et al. [[47]](#_bookmark45) that abscess is associated with inflammatory processes capable of producing ~100 mM of H2O2 (ROS). Alterations in the anti-oxidant enzymes and lipid per- oxidation status of the treated rats compared to the negative control further supported the cellular inflammatory actions.

The liver in mammalian systems is prone to drug-induced

injury due to its central role in drug metabolism, detoxifica- tion, storage, and portal location within the circulation [[48]](#_bookmark46). It is the most sensitive predictor of chemical toxicity that cor- relates well with histopathology and serum biochemistry with little inter-animal variations [[19]](#_bookmark25). As the major site of AL, ABZ and IVR metabolism [[25,49,50]](#_bookmark29), the liver may be prone to the toxic effects of these drugs and or their metabolites. Cell membrane damage in the liver is usually associated with the release of a number of cytoplasmic enzymes into the circu- latory system; this provides the basis for clinical diagnosis. Serum AST and ALT are the most used biochemical markers of hepatocellular necrosis and are considered sensitive in- dicators of hepatic drug induced injuries [[51,52]](#_bookmark49). Significant increase in the activities of serum ALT, AST and total bilirubin observed in the treated rats mostly from groups D, E, G, H, J, K and L suggests acute hepatocellular injury due to drug induced necrosis. It is suggested that the administered drugs and or their metabolites induced lipid peroxidative damage to the hepatocytes (accidental cell death; necrosis) which increased cell membrane permeability to the cellular enzymes. This is supported by the observation of numerous hepatic necroses in the liver cells and significant increase in serum lipid peroxi- dation in the treated rats. Moreover the reports that Iver- mectin [[29]](#_bookmark31), Abendanzole [[25]](#_bookmark29), Artemether-Lumefantrine [[49]](#_bookmark47) and co-administration of albendazole and ivermectin [[50]](#_bookmark48) treated rats at different concentrations significantly

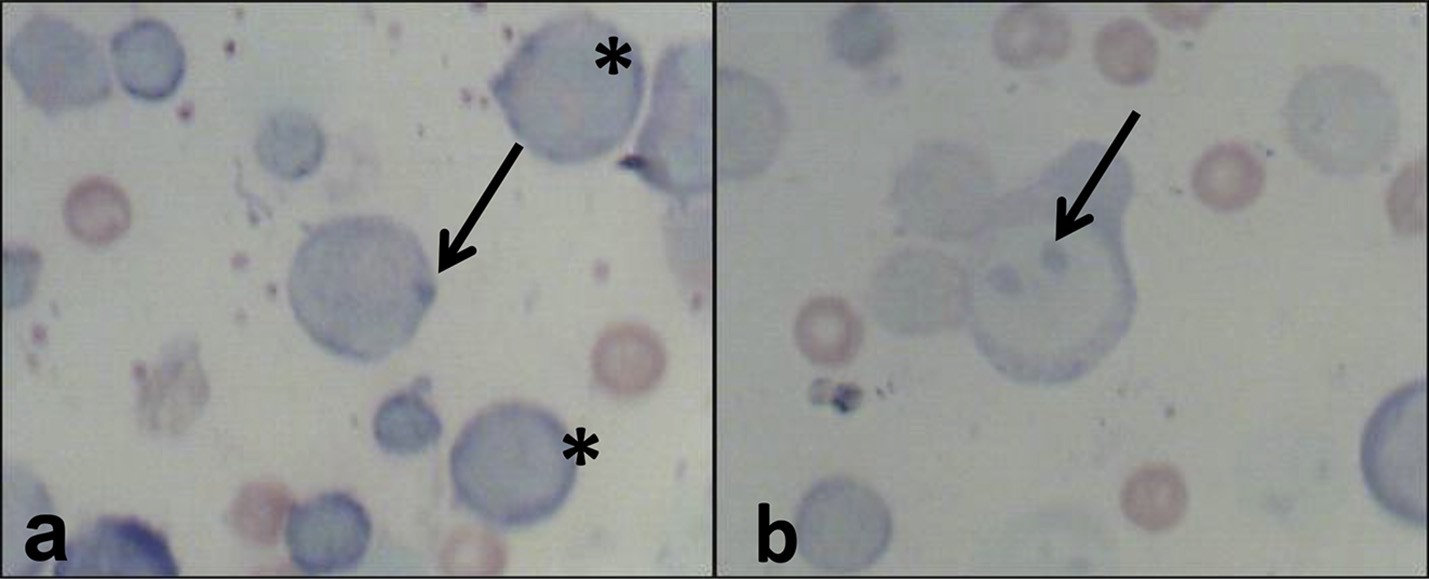


Fig. 4 e (aeb): Asterisks showed normal polychromatic erythrocytes while arrows showed micronucleated polychromatic erythrocytes from bone marrow cells of rats.

116 [e gypti an j o ur nal o f b a sic and a p p l i ed sci e n c e s 2 ( 201 5 ) 110](http://dx.doi.org/10.1016/j.ejbas.2015.03.001) e[119](http://dx.doi.org/10.1016/j.ejbas.2015.03.001)

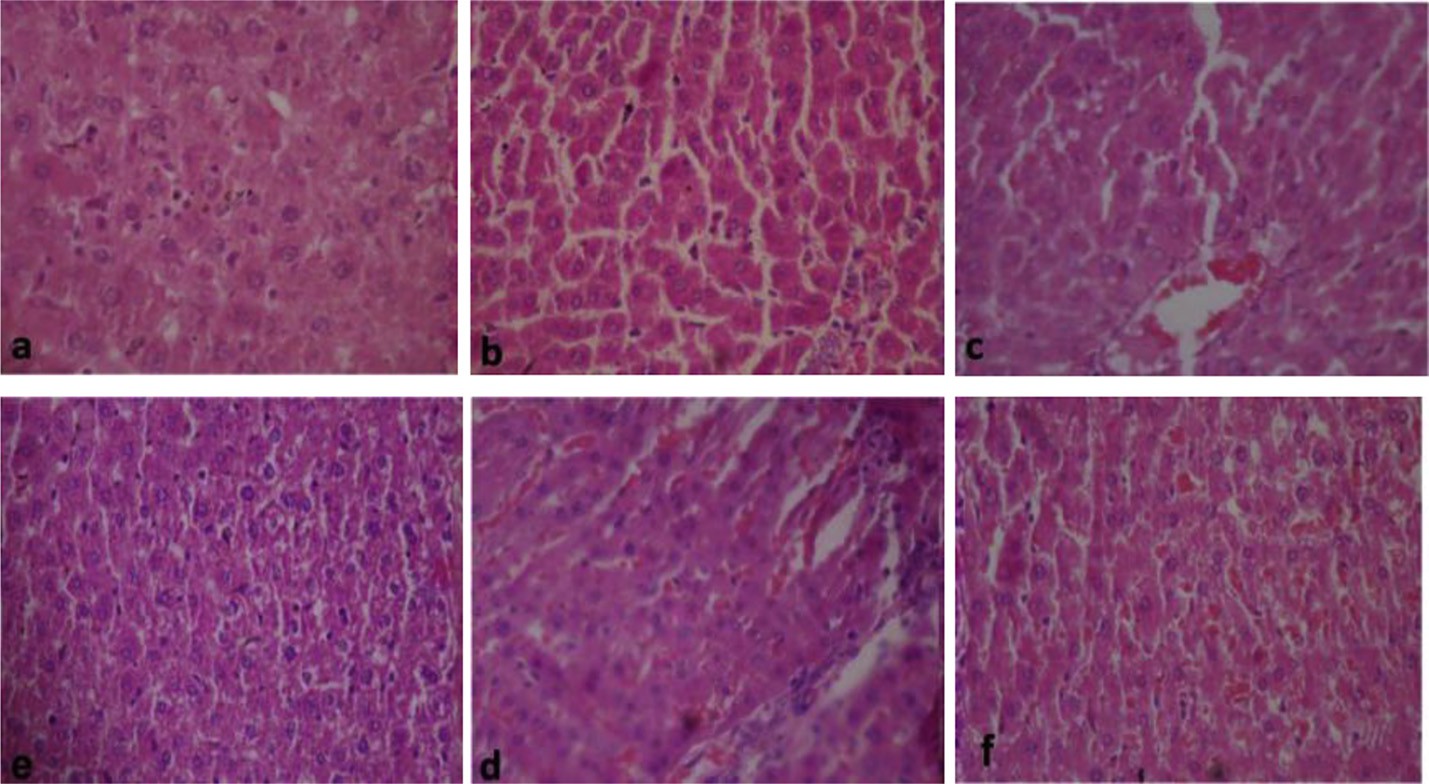


Fig. 5 e Liver tissues of rats exposed to drug combinations (H&E, x 400). (a) Section of liver from rat in the negative control group showing relatively normal hexagonal or pentagonal lobule of hepatocytes. (b) Single cell hepatocellular necrosis and kupffer cell hyperplasia (c) Multiple foci vacuolar changes and congestion of the sinusoids. (d) Thinning of hepatic cord and single cell hepatocellar necrosis. (e) Vacuolar changes in the hepatocytes and mild congestion of the sinusoids. (f) Severe congestion of the sinusoids by inflammatory cells.

increased transaminase concentrations is in concert with the observations herein. Also, increase in serum total bilirubin concentration with concomitant elevation of transaminases in AL treated rats have been associated with hepatocellular injury [[53,54]](#_bookmark51). Histological study yields the most reliable in- formation on the type of lesions induced by drugs and their metabolites in tissues. It is also useful in providing informa- tion about acute and chronic effects of toxic substances that may not be detected by biochemical analysis [[55,56]](#_bookmark52). Acute exposure of the drug combinations induced hepatic necrosis, kupffer cell hyperplasia and congestions of the hepatocytes by inflammatory cells observed in the liver cells of the treated rats correlated with the clinical signs and biochemical find- ings. These lesions were similarly reported in rats dosed with combination of Artemether-Lumefantrine and were attrib- uted to drug induced liver injury [[57]](#_bookmark53).

Drug combinations can act in synergy, potentiation and or

antagonism to induce alterations in enzyme biochemistry, biological molecules (ROS) and cell membrane [[20,58,59]](#_bookmark26). These alterations may include increase in cellular formation of oxidative stress via creating imbalance between ROS and the antioxidant systems [[48]](#_bookmark46). SOD activity is important in preventing oxidative damage by scavenging and converting superoxide anions to hydrogen peroxides, while catalase causes the decomposition of the hydrogen peroxide to protect tissues from the actions of the highly reactive hydroxyl radi- cals [[52,60]](#_bookmark50). During these processes the activities of these en- zymes may be altered leading to ROS induced pathological disorders and DNA damage [[46]](#_bookmark44). The observed significant decrease in the activities of SOD and CAT in the treated rats suggests harmful effects of the drug combinations (groups D, E, G, H, J, K and L) via excess undetoxified free radical forma- tion. These unscavenged free radicals probably induced lip- operoxide formation which caused lipid peroxidation in the cell membrane (increase MDA concentration) of the treated

rats. Studies using rats exposed to AL [[49,53]](#_bookmark47) and ABZ [[61]](#_bookmark54), and rabbits treated with IVR [[62,63]](#_bookmark55) showed reduced SOD and CAT activities either in the serum or liver with concomitant in- crease in MDA concentrations, are in concert with the findings herein.

Micronucleus (MN) test is the most widely utilized and recommended test for the genotoxic and mutagenic evalua- tion of chemicals [[64,65]](#_bookmark56). This is due to its technical simplicity and ability to detect both clastogens and aneugens [[64]](#_bookmark56). The

significant increase in the MNPCE observed in bone marrow of rats treated with ×2.0 AL, AL + ABZ, ×2.0 AL + ABZ, AL + IVR,

×2.0 AL + IVR in groups D, E, G, H and J respectively suggests

DNA damage induced by the tested drug combinations. It is plausible that the drug combinations induced MNPCE was via ROS formation. Since DNA damage may be associated with accidental cell death (necrosis) or apoptosis induced by reac- tive oxygen species [[66]](#_bookmark57). The combination of these drugs possibly affected the actively proliferative bone marrow cells during the M-phase of the cell cycle to induce oxidative DNA damage in the treated rats. Similar data obtained from the cyclophosphamide treated rats (positive control; group M) is in support of this assertion. Cyclophosphamide, an alkylating agent widely used in cancer chemotherapy [[67]](#_bookmark58), is known to alkylate DNA and protein via oxidative stress induction [[68,69]](#_bookmark59), leading to cytotoxicity and genotoxicity [[68]](#_bookmark59). Alter- ations in the serum oxidative stress parameters and increase MNPCE formation in the bone marrow of cyclophosphamide treated rats (positive control; group M), validated the findings herein. Studies similarly reported that mice treated with abendazole harbored significant increase MNPCE in the bone marrow [[70]](#_bookmark60). Pediatric patients with hepatic hydatid disease on abendazole treatment presented significant increase in sister chromatid exchange (SCE) and micronucleus fre- quencies in peripheral lymphocytes [[39]](#_bookmark39). Also, mice exposed to ivermectin expressed higher chromosome aberration,

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MNPCE formation and DNA fragmentation compared to the negative control [[20]](#_bookmark26). The combinations of the tested drugs generated reactive oxygen species (ROS) which played important role in MNPCE formation in the treated rats. So- matic DNA damage due to drug toxicity predisposes cells to chromosome related disorders, aging and carcinogenicity [[29,39,59,71,72]](#_bookmark31).

The role of oxidative stress in the induction of abnormal cellular functions and pathological disorders are the current research focus for many studies [[73](#_bookmark61)e[75]](#_bookmark61). Oxidative stress induced cell damage may occur via a number of mecha- nisms [[74]](#_bookmark62) and the damaged cells may be eliminated either by programmed cell death (apoptosis) or accidental cell death (necrosis). Necrosis, the most common histological lesions observed in the liver of the treated rats, is associated with the destruction of certain signal pathways in the cells and the disruption of mitochondrial functions via in- flammatory process [[76]](#_bookmark63). Cellular necrosis and inflammatory cells have been associated with liver damage in Artemether- Lumefantrine treated rats [[57]](#_bookmark53). This study showed that the combination of the tested drugs, mostly at higher doses elicited ROS formation in rats. Oxidative stress in- duction correlated with cellular necrosis, inflammatory cell production and other pathological lesions observed in the hepatocytes. Also MNPCE formation in bone marrow cells

of rats. In conclusion, A + L, AL + ABZ, and AL + IVR

drug combinations induced hepatotoxicity and somatic mutation in rats may be associated with oxidative stress induction.

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## referen c es

1. [International Agency for Research on Cancer (IARC). IARC](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref1) [monograph on the evaluation of carcinogenic risks to](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref1) [humans. Schistosomes, liver flukes and *Helicobacter pylori*,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref1) [vol. 61; 1994. Lyon](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref1).
2. [Greenwood B, Mutabingwa T. Malaria in 2002. Nature](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref2) [2002;415:670](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref2)e[2](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref2).
3. [World Health Organization (WHO). Prevention and control of](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref3) [schistosomiasis and soil transmitted Helminthiasis. Fourth](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref3) [Report of the expert committee. Technical report series](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref3) [No712. WHO Geneva; 2002](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref3).
4. [Raso G, Luginbuhl A, Adjoua CA, Tian-Bi NT, Silue KD.](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref4) [Multiple parasite infections and their relationship to self-](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref4) [reported morbidity in a community of rural Co^te d'Ivoire. Int](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref4) [J Epidemiol 2004;33:1092](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref4)e[102](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref4).
5. [World Health Organization (WHO). Towards accelerated](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref5) [reduction of neglected tropical diseases. Geneva,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref5) [Switzerland: World Health Organization; 2012](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref5).
6. [Amin AA, Kokwaro GO. Antimalarial drug quality in Africa. J](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref6) [Clin Pharmacol Ther 2007;32:429](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref6)e[40](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref6).
7. Premji ZG. Coartem[®: the journey to the clinic. Malar J](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref7) [2009;8:1](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref7)e[6](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref7).
8. [World Health Organization (WHO). Guidelines for the](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref8) [treatment of malaria. 2nd ed. Geneva, Switzerland: World](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref8) [Health Organization; 2010](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref8).
9. [Kurth F, Belard S, Adegnika AA, Gaye O, Kremsner PG,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref9) [Ramharter M. Do paediatric drug formulations of artemisinin](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref9) [combination therapies improve the treatment of children](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref9) [with malaria? A systematic review and meta-analysis.](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref9) [Lancet Infect Dis 2010;10:125](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref9)e[32](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref9).
10. [Ngasala BE, Malmberg M, Carlsson AM, Ferreira PE,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref10)

[Petzold MG, Blessborn D, et al. Efficacy and effectiveness of](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref10) [artemether-lumefantrine after initial and repeated](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref10) [treatment in children <5 years of age with acute](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref10) [uncomplicated *Plasmodium falciparum* malaria in rural](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref10) [Tanzania: a randomized trial. Clin Infect Dis 2011;52:873](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref10)e[82](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref10).

1. [Nosten F, White NJ. Artemisinin-Based combination](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref11) [treatment of falciparum malaria. Am J Trop Med Hyg](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref11) [2007;77:181](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref11)e[92](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref11).
2. [Campbell WC. Benzimidazoles: veterinary uses. Parasitol](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref12) [Today 1990;6:130](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref12)e[3](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref12).
3. [Gann PH, Neva FA, Gam AA. A randomized trial of single-](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref13) [and two-dose ivermectin versus thiabendazole for the](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref13) [treatment of strongyloidiasis. J Infect Dis 1994;169:1076](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref13)e[9](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref13).
4. [Geary TG. Ivermectin 20 years on: maturation of a wonder](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref14) [drug. Trends Parasitol 2005;21:530](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref14)e[2](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref14).
5. [Horton J, Witt C, Ottesen EA, Lazdins JK, Addiss DG. An](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref15) [analysis of the safety of single dose, two drug regimens used](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref15) [in programmes to eliminate lymphatic filariasis. Parasitology](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref15) [2000;121:S147](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref15)e[60](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref15).
6. [Mohammed KA, Haji HJ, Gabrielli A, Mubila L, Chitsulo L,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref16) [Bradley MH, et al. Triple co-administration of ivermectin,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref16) [albendazole and praziquantel in Zanzibar: a safety study.](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref16) [PLoS Neglect Trop Dis 2008;2:1](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref16)e[7](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref16).
7. [Adjei GO, Goka BQ, Binka F, Kurtzhals JA. Artemether-](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref17) [lumefantrine: an oral antimalarial for uncomplicated](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref17) [malaria in children. Exptal Rev Anti Infect Ther](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref17) [2009;7:669](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref17)e[81](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref17).
8. [Rang HP, Dale MM. Pharmacology. 2nd ed. Longman Goup,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref18) [UK Limited; 1991](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref18).
9. [Sturgill MG, Lambert GH. Xenobiotic-induced hepatotoxicity:](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref19) [mechanisms of liver injury and methods of monitoring](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref19) [hepatic function. Clin Chem 1997;43:1512](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref19)e[26](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref19).
10. [Essa BH, El-Nahas AF, Mahrous UE, El-Tahawy AS.](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref20) [Genotoxicity of Ivermectin (P-gp inhibitor) as a model of](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref20) [drug-drug interaction. Alex J Vet Sci 2012;38:23](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref20)e[32](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref20).
11. [Brewer TG, Peggins JO, Grate SJ, Petras JM, Levine BS,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref21)

[Weina PJ, et al. Neurotoxicity in animals due to arteether and](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref21) [artemether. Trans R Soc Trop Med Hyg 1994;88:S33](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref21)e[6](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref21).

1. [Nontprasert A, Pukrittayakamee S, Dondorp AM, Clemens R,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref22) [Looareesuwan S, White NJ. Neuropathologic toxicity of](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref22) [artemisinin derivatives in a mouse model. Am J Trop Med](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref22) [Hyg 2002;67:423](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref22)e[9](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref22).
2. [Petras JM, Kyle DE, Gettayacamin M, Young GD, Bauman RA,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref23) [Webster HK, et al. Arteether: risks of two week](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref23) [administration in *Macaca mulatta*. Am J Trop Med Hyg](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref23) [1997;56:390](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref23)e[6](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref23).
3. [Alc](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref24)a^[ntara DD, Ribeiro HF, Cardoso PC, Arau´ jo TM,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref24)

[Burbano RR, Guimar](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref24)a~[es AC, et al. In vitro evaluation of the](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref24) [cytotoxic and genotoxiceffects of artemether, an](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref24) [antimalarial drug, in a gastric cancer cell line (PG100). J Appl](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref24) [Toxicol 2013;33:151](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref24)e[6](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref24).

1. [Abd El-Rahman MA, Abdel-Nabi IM, Omran MA,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref25)

[Mohamed MF. Cytotoxic effects of Albendazole, antiparasitic](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref25) [drug, on the liver of the rat: sub-chronic study. Egypt J Biol](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref25) [1999;1:16](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref25)e[29](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref25).

1. [Molinari G, Sloneski S, Reigosa MA, Larramendy ML. *In vitro*](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref26)

[genotoxic and cytotoxic effects of ivermectin and its](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref26)

118 [e gypti an j o ur nal o f b a sic and a p p l i ed sci e n c e s 2 ( 201 5 ) 110](http://dx.doi.org/10.1016/j.ejbas.2015.03.001) e[119](http://dx.doi.org/10.1016/j.ejbas.2015.03.001)

[formulation ivomec on Chinese hamster ovary (CHOK1) cells.](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref26) [J Hazard Mater 2009;15:1074](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref26)e[82](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref26).

1. [Abolaji AO, Eteng MU, Omonua O, Adenrele Y. Influence of](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref27) [co-administration of artemether and lumefantrine on](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref27) [selected plasma biochemical and erythrocyte oxidative](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref27) [stress indices in female Wistar rats. Hum Exp Toxicol](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref27) [2013;32:206](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref27)e[15](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref27).
2. [Lin Y, Chern H, Chu M. Hepatotoxicity in the review of](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref28) [clinical safety data. Drug Info J 2003;37:155](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref28)e[8](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref28).
3. [Qureshi S. Biochemical toxicity of Ivermectin in Wistar](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref29) [albino rats. Ame Eura J Toxicol Sci 2013;5:15](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref29)e[9](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref29).
4. [Aebi H. Catalase estimation. In: Bergmeyer HV, editor.](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref30) [Methods of enzymatic analysis. New York: Verlag Chemic;](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref30) [1974](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref30).
5. [Magwere T, Naik YS, Hasler JA. Effects of chloroquine](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref31) [treatment on antioxidant enzymes in rat liver and kidney.](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref31) [Free Radic Biol Med 1997;22:321](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref31)e[7](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref31).
6. [Nichaus WG, Samuelson B. Formation of malondialdehyde](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref32) [from phospholipid arachidonate during microsomal lipid](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref32) [peroxidation. Eur J Biochem 1968;6:126](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref32)e[30](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref32).
7. [Lowry OH, Rosebrought NJ, Farr AL, Randall RJ. Protein](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref33) [measurement with the folin phenol reagent. J Biol Chem](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref33) [1951;193:265](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref33)e[75](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref33).
8. [Reitman S, Frankel S. A colorimetric method for](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref34) [determination of serum glucose oxaloacetate and glutamic](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref34) [pyruvate transaminases. Am J Clin Pathol 1957;28:53](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref34)e[6](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref34).
9. [Treitz W. Fundamentals of clinical chemistry with clinical](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref35) [correlation. W.B. Saunders; 1970. Philadelphia vary B.](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref35) [detaeselier](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref35).
10. [Schmid W. The micronucleus test. Mutat Res 1975;31:9](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref36)e[15](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref36).
11. [Adams HR. Chemotherapy of parasitic diseases. In:](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref37) [Veterinary pharmacology and therapeutics. 7th eds. Ames,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref37) [Iowa 50014: Iowa state University Press; 1995. p. 898](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref37).
12. [Dadarkar SS, Deore MD, Gatne MM. Comparative evaluation](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref38) [of acute toxicity of ivermectin by two methods after single](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref38) [subcutaneous administration in rats. Regul Toxicol](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref38) [Pharmacol 2007;47:257](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref38)e[60](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref38).
13. [Oztas S, Salman AB, Tatar A, Yigiter M, Yazgi H, Ertek M,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref39) [et al. Genotoxic effect of albendazole in pediatric patients](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref39) [with hepatic hydatid disease. Int J Infect Dis 2007;11:446](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref39)e[9](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref39).
14. [Cullen JM. Mechanistic classification of liver injury. Toxicol](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref40) [Pathol 2005;33:6](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref40)e[8](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref40).
15. [Kamgno J, Gardon J, Gardon-Wendel N, Ngangue D,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref41)

[Duke BOL, Boussinesq M. Adverse systemic reactions to](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref41) [treatment of onchocerciasis with ivermectin at normal and](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref41) [high doses given annually or three monthly. Trans R Soc](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref41) [Trop Med Hyg 2004;98:496](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref41)e[504](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref41).

1. [Saprsa A, Bonnetblance JM, Peyrot I, Loustaud-Ratti V,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref42) [Vidal E, Bedane C. Systemic adverse reactions with](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref42) [Ivermectin treatment of scabies. Ann Dermatol Venereol](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref42) [2006;133:784](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref42)e[7](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref42).
2. [Njoo FL, Hack CE, Oosting J, Stilma JS, Kijlstra A. Neutrophils](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref43) [activation in Ivermectin-treated onchocerciasis patients.](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref43) [Clin Exp Immunol 1993;94:330](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref43)e[3](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref43).
3. [Tischendorf FW, Brattig NW, Hoyer A, Medina-De La GCE,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref44) [Geisinger F. Modulatory effects of antifilial drugs ivermectin,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref44) [CGP 6140 and CGP 20376 on the oxidative burst of](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref44) [eosinophilic granulocytes. Acta Trop 1993;53:27](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref44)e[37](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref44).
4. [Jaeschke H. Reactive oxygen species and mechanisms of](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref45) [inflammatory liver injury. J Gastroenterol Hepatol](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref45) [2000;15:718](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref45)e[24](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref45).
5. [Ramaiah S, Jaeschke H. Role of neutrophils in the](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref46) [pathogenesis of acute inflammatory liver injury. Toxicol](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref46) [Pathol 2007;35:757](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref46)e[66](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref46).
6. [Hyslop PA, Hinshaw DB, Scraufstatter IU, Cochrane CG,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref47) [Kunz S, Vosbeck K. Hydrogen peroxide as a potent](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref47) [bacteriostatic antibiotic: implications for host defense. Free](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref47) [Radic Biol Med 1995;19:31](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref47)e[7](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref47).
7. [Jones AL. Anatomy of the normal liver. In: Zakin D, Boyer TD,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref48) [editors. Hepatology: a textbook of liver disease. 3rd ed.](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref48) [Philadelphia: WB Saunders; 1996](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref48).
8. [Adaramoye OA, Osaimoje DO, Akinsanya AM, Nneji CM,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref49) [Fafunso MA, Ademowo OG. Changes in antioxidant status](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref49) [and biochemical indices after acute administration of](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref49) [Artemether, Artemether-Lumefantrine and Halofantrine in](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref49) [rats. Basic Clin Pharmacol Toxicol 2008;102:412](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref49)e[8](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref49).
9. [Arise RO, Malomo SO. Effects of ivermectin and albendazole](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref50) [on some liver and kidney function indices in rats. Afri J](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref50) [Biochem Res 2009;3:190](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref50)e[7](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref50).
10. [Choi GY, Yang HW, Cho SH, Kang DW, Go H, Lee WC, et al.](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref51) [Acute drug-induced hepatitis caused by albendazole. J](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref51) [Korean Med Sci 2008;23:903](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref51)e[5](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref51).
11. [Timbrel JA. Principles of biochemical toxicology. 4th ed. New](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref52) [York, USA: Informa Healthcare; 2009](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref52).
12. [Anyasor GN, Odunsanya OT. Coartemether in dietary oil](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref53) [induces oxidative stress and hepatotoxicity in albino rat.](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref53) [Researcher 2011;3:35](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref53)e[41](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref53).
13. [Giannini E, Testa R, Savarino V. Liver enzyme alteration: a](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref54) [guide for clinicians. Can Med Assoc J 2005;172:367](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref54)e[79](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref54).
14. [Amacher DE, Schomaker SJ, Boldt SE, Mirsky M. The](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref55) [relationship among microsomal enzyme induction, liver](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref55) [weight and histological change in Cynomolagus monkey](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref55) [toxicology studies. Food Chem Toxicol 2006;44:528](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref55)e[37](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref55).
15. [Lanning LL, Creasy DM, Chapin RE, Mann PC, Barlow NJ,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref56) [Regan KS, et al. Recommended approaches for the](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref56) [evaluation of testicular and epididymal toxicity. Toxicol](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref56) [Pathol 2002;30:507](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref56)e[20](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref56).
16. [Ukekwe IF, Akali PA, Ezike AC, Okoli CO. Assessment of the](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref57) [sub-acute and delayed toxicity of Artemether-Lumefantrine](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref57) [combination in rats. Int J Res Ayurveda Pharm 2013;4:168](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref57)e[76](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref57).
17. [Davies DT. Enzymology in preclinical safety evaluation.](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref58) [Toxicol Pathol 1992;20:501](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref58)e[5](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref58).
18. [Escobar-Garcia D, Camacho-Carranza R, Perez I, Dorado V,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref59) [Arriaga-Alba M, Espinosa-Aguirre JJ. S9 indution by the](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref59) [combined treatment with cyclohexanol and albendazole.](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref59) [Mutagenesis 2001;16:523](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref59)e[8](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref59).
19. [Michiels C, Raes M, Toussaint O, Remacle J. Importance of](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref60) [Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref60) [survival against oxidative stress. Free Radic Biol Med](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref60) [1994;17:235](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref60)e[48](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref60).
20. [Yarsan E, Ceik S, Eraslan G, Aycicek H. Effects of albendazole](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref61) [treatment on lipid peroxidation of healthy and *Toxocaris canis*](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref61)[infected mice. Isr Vet Med Assoc J 2002;57:1](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref61)e[11](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref61).
21. [Atakisi E, Atakisi O, Topcu B, Uzun M. Effect of therapeutic](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref62) [dose of ivermectin on plasma nitric oxide and total](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref62) [antioxidant capacity in rabbits. Euro Rev Med Pharmacol Sci](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref62) [2009;13:425](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref62)e[9](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref62).
22. [Behera SK, Dimri U, Singh SK, Mohanta RK. The curative and](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref63) [antioxidative efficiency of ivermectin and ivermectin plus](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref63) [vitamin E and selenium treatment on canine *Sarcoptes scabiei*](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref63)[infestation. Vet Res Commun 2011;35:237](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref63)e[44](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref63).
23. [Krishna G, Hayashi M. In vivo rodent micronucleus assay:](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref64) [protocol, conduct and data interpretation. Mutat Res](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref64) [2000;455:155](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref64)e[66](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref64).
24. [Morita T, Asano N, Awogi T, Sasaki YF, Sato S, Shimada H,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref65) [et al. Evaluation of the rodent micronucleus assay in the](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref65) [screening of IARC carcinogens (Group 1. 2A and 2B). The](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref65) [summary report of the 6th collaborative study by CSGMT/](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref65) [JEMS MMS. Mutat Res 1997;389:3](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref65)e[122](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref65).
25. [Ouanes Z, Abid S, Ayed I, Anane R, Mobio T, Creppy EE, et al.](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref66) [Induction of micronuclei by Zearalenone in Vero monkey](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref66) [kidney cells and in bone marrow cells of mice: protective](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref66) [effect of vitamin E. Mutat Res 2003;538(1](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref66)e[2):63](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref66)e[70](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref66).
26. [Fleming RE. An overview of cyclophosphamide and](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref67) [ifosfamide pharmacology. Pharmacotherapy](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref67) [1997;17:1465](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref67)e[545](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref67).

[e g ypti an j o ur nal o f b a sic and a pp l i e d sci en c e s 2 ( 201 5 ) 110](http://dx.doi.org/10.1016/j.ejbas.2015.03.001) e[119](http://dx.doi.org/10.1016/j.ejbas.2015.03.001) 119

1. [Chakraborty P, Sk UH, Murmu N, Das JK, Pal S,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref68) [Bhattacharya S. Modulation of cyclophosphamide-induced](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref68) [cellular toxicity by diphenylmethyl selenocyanate *in vivo*, an](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref68) [enzymatic study. J Cancer Mol 2009;4:183](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref68)e[9](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref68).
2. [Ghosh D, Das UB, Ghosh S, Mallick M, Debnath J. Testicular](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref69) [gametogenic and steroidogenic activities in](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref69) [cyclophosphamide treated rat: a correlative study with](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref69) [testicular oxidative stress. Drug Chem Toxicol](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref69) [2002;25:281](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref69)e[92](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref69).
3. [Alkan FU, Sener S. Lack of the antimutagenic effect of](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref70) [ascorbic acid on the genotoxicity of albendazole in mouse](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref70) [bone marrow cells. Bull Vet Inst Pulawy 2009;53:493](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref70)e[7](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref70).
4. [Brozovic G, Orsolic N, Rozgaj R, Kasuba V, Knezevic F,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref71) [Knezevic AH, et al. DNA damage and repair after exposure to](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref71) [sevoflurane *in vivo*, evaluated in Swiss albino mice by the](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref71) [alkaline comet assay and micronucleus test. J Appl Genet](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref71) [2010;51:79](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref71)e[86](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref71).
5. [Van Schooten FJ, Besarati Nia A, De Flora S, D'Agostini F,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref72) [Izzotti A, Camoirano A, et al. Effects of oral administration](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref72) [of N-acetyl-L-cysteine: a multi-biomarker study in](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref72) [smokers. Cancer Epidemiol Biomarkers Prev](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref72)

[2002;11:167](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref72)e[75](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref72).

1. [Halliwell B. Role of free radicals in the neurodegenerative](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref73) [diseases: therapeutic implications for antioxidant treatment.](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref73) [Drugs Aging 2001;18:685](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref73)e[716](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref73).
2. [Jomova K, Vondrakova D, Lawson M, Valko M. Metals,](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref74) [oxidative stress and neurodegenerative disorders. Mol Cell](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref74) [Biochem 2010;345:91](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref74)e[104](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref74).
3. [Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. Role of](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref75) [oxygen radicals in DNA damage and cancer incidence. Mol](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref75) [Cell Biochem 2004;266:37](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref75)e[56](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref75).
4. [Pulido MD, Parrish AR. Metal-induced apoptosis:](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref76) [mechanisms. Mutat Res 2003;533:227](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref76)e[41](http://refhub.elsevier.com/S2314-808X(15)00017-2/sref76).