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EFFECT OF COMBINED ADMINISTRATION OF ARTEQUIN® AND PEFLOXACIN ON SOME INDICES OF LIVER AND RENAL FUNCTIONS OF MALE ALBINO WISTAR RATS

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Abstract

Co-infection with malaria and typhoid fever is a common presentation in many endemic regions of the world including Nigeria. Affected persons are often treated by co-administration of suitable antibacterial and anti-malarial agents. The present study investigated the effect of simultaneous administration of pefloxacin (a second generation quinolone antibiotic) and artequin® (an artemisinin combination anti-malarial drug) on some indices of renal and liver functions in rats. Twenty male albino rats were randomly assigned into four groups A, B, C and D. Animals in Group A served as control. Group B and C were treated with therapeutic doses of pefloxacin (6. 67 mg per kg bw twice daily) and artequin® (5 mg of mefloquin and 4 mg of artesunate per kg bw) respectively. Group D received the two drugs simultaneously. The results show that drug administration induced significant increase ($p < 0.05$) in the serum activity of ALT, AST and ALP. Serum creatinine and urea also increased significantly ($p< 0.05$) compared to control. There was no significant increase in the level of Na^+ in serum ($p> 0.05$) whereas K^+, Cl^- and HCO_3 increased significantly ($p< 0.05$). Histological assessment of the liver revealed areas of cellular degeneration, vascular congestion and pyknotic nuclei. The kidney showed areas of inflammation, cellular distortion and pyknotic nuclei. Generally, the observed changes in the biochemical indices of liver and kidney functions as well as histopathology are manifestations of moderate hepatotoxicity and nephropathy precipitated by drug administration.

Keywords: Artequin, Pefloxacin, Hepatoxicity, Nephrotoxicity, Serum Enzymes.

Introduction

Malaria and typhoid fever are among the most prevalent tropical diseases. Malaria alone is estimated to have an annual incidence of 124 to 283 million worldwide [1] while typhoid fever has been reported to be responsible for about 200,000 deaths annually worldwide [2]. An association between malaria and typhoid fever was first described in medical literature in the middle of the 19th century and was named typhomalarial fever by United State Government [3]. This has been confirmed by other authors [4][5][6]. It has also been postulated that malaria can progress to typhoid or that malaria always co-infect with typhoid/paratyphoid [7].

Malaria and typhoid fever are caused by different organisms, *Plasmodium falciparium* (a protozoan) and *Salmonella typhi* (a Gram negative bacillus) respectively, transmitted via different mechanisms, both diseases share similar symptoms [8]. Individuals in areas endemic for both malaria and typhoid are at substantial risk for contracting both diseases either concurrently or an acute infection superimposed on a chronic one [9]. In the treatment of persons co-infected with malaria and typhoid fever, clinicians are faced with the challenge of choosing a suitable anti-malarial drug and antibiotics from an available list of therapeutic options. Currently, the World Health Organisation (WHO) recommends artemisinin-base combination therapies (ACTs) as first line therapy for malaria while the flouroquinolones are best suited for the treatment of typhoid due to the development of resistance to other antibiotics [10]. These drugs have been proven to be very effective against the pathogenic microorganisms. However, the toxicities of flouroquinolones and artequin® have been reported independently, even at normal therapeutic doses [11][12][13][14]. There are reports indicating that malaria and typhoid are being over-diagnosed and over-treated in some endemic areas of the world thereby increasing further the risk of drug induced toxicity [5].

In view of the fact that the liver and kidney are major organs involved in drug metabolism, and are highly susceptible to the deleterious effects of xenobiotics, the present study was carried out to assess the effect of co-administration of pefloxacin and artequin® on the integrity of liver and kidney. Parameters studied included serum enzymes (ALT, AST and ALP), serum electrolytes (sodium, potassium, chloride, bicarbonate), creatinine, urea as well as histopathological examination of tissue samples.

Methods and Materials

Artequin® (600/750 mg) and Pefloxacin (400 mg) were obtained from the Pharmacy unit of the University of Uyo Teaching Hospital, Uyo, Nigeria.

Experimental Design

Twenty (20) mature male albino Wistar rats weighing between 180 – 240 g were purchased from the College of Health Science Animal House, University of Uyo, Uyo, Nigeria. The animals were housed in cages and allowed to acclimatise before commencement of experiment. They were maintained under hygienic and favourable conditions under a 12 hour light / 12 hour dark cycle with feeds and water provided *ad libitum.*

The animals were randomly grouped into four groups with five animals per group:

Group A: control, fed with rat pellet and water only.

Group B: received therapeutic dose of pefloxacin (6. 67 mg/kg body weight twice daily).

Group C: received therapeutic dose of artequin® (5 mg of mefloquin and 4 mg of artesunate per kg bw)

Group D: received therapeutic doses of both drugs (6. 67 mg of perfloxacin per kg body weight twice daily, 5 mg of mefloquin and 4 mg of artesunate per kg bw). Artequin® was administered for three days while administration of pefloxacin continued for ten days based on the normal therapeutic regimen for these drugs.

At the end of the experimental period, the animals were sacrificed under chloroform anaesthesia. Blood samples were obtained by cardiac puncture using syringe and needle. The blood was transferred into anticoagulant free sample bottles and serum was obtained by centrifugation at 3000 rpm for ten minutes. Liver samples were also excised, preserved in buffered formalin and used for histological studies.

Estimation of Biochemical Parameters

Estimation of serum enzymes (ALT, AST and ALP), serum electrolytes (sodium, potassium, chloride, bicarbonate), creatinine and urea were carried out according to manufacturer's instructions as contained in the relevant assay kits.

Histopathological Studies

Three animals were selected randomly from each group and dissected through a central abdominal incision. The livers and kidneys were harvested. Organ sections were passed through the processes of fixation, dehydration, clearing, infiltration, embedding, sectioning and staining with haematoxylin and eosin (H and E) for examination under a light microscope. Photomicrographs of some

of the tissue sections were taken using a digital camera fitted to the light microscope at a magnification of 400X.

Statistical Analysis of Data

The data obtained were expressed as mean ± SEM. The one way analysis of variance (ANOVA) was used for comparison and results were subject to post hoc test using Turkey multiple comparison. Test values of $p< 0.05$ were considered significant.

Results

The results of the effect of therapeutic doses of pefloxacin and artequin® (single and combined administration) on liver enzymes of male albino Wistar rats are presented in Table 1. Although slight increases were observed in the ALT levels of rats administered artequin® and pefloxacin separately, these changes were not significant ($p>0.05$) when compared to the control. However, the group co-administered artequin® and pefloxacin demonstrated significant increase from 47.00 ± 1.22 U/L (control) to 52.20 ± 0.80 U/L (Group 4). Further comparison showed that the values of ALT in Group 4 were significantly different from Group 2 (49.00 ± 0.89 U/L) and Group 3 (49.02 ± 0.81 U/L). The values of AST for the treated groups were observed to be significantly increased when compared to the control though the effect of artequin® (172.20 ± 0.80 U/L) was significantly lower than the effect of pefloxacin (177.20 ± 0.97 U/L) at $p<0.05$. ALT was also significantly increased in the treated groups ($p< 0.05$).

Table 2 shows the effect of drug administration on some indices of renal function in the experimental animals. There were no significant changes ($p>0.05$) in concentrations of sodium in serum. In the case of potassium ions, it was observed that serum concentrations decreased significantly ($p< 0.05$) as a result of drug administration. Artequin® was found to precipitate a significant increase ($p<0.05$) in concentration of chloride ions whereas pefloxacin did not cause any significant increase ($p>0.05$) in chloride ion levels. Animals in group D which received artequin® and pefloxacin simultaneously demonstrated a significant increase ($p<0.05$) in the levels of chloride ions. A similar significant increase ($p<0.05$) in serum bicarbonate levels occurred sequel to drug administration. Table 2 also shows that serum creatinine increased significantly ($p<0.05$) in all treatment groups compared to control. However, the increase in serum urea was significant ($p<0.05$) only in the group co-administered the two drugs.

Discussion

The liver is an organ that plays a central role in the metabolism of xenobiotics. Consequently, it is highly susceptible to injury from drugs and other foreign substances [15]. Drug induced liver injury (DILI) is reported to be a leading cause of acute liver failure and reason for regulatory actions against previously approved drugs [16]. Toxic injury to the liver can be assessed by the determination of serum levels of enzymes such as ALT, AST and ALP. Elevations in the activities of these enzymes in the serum serve as positive indicators of liver damage because these enzymes leak into circulation as a consequence of hepatocellular damage [17]. In the present study, the activities of ALT, AST and ALP increased significantly as a consequence of drug administration. This was particularly evident when the two drugs were co-administered, indicating damage to the hepatocytes. Ait-kaled et al [18] observed that pefloxacin is safe and highly effective in the treatment of typhoid fever. However, a more recent study [19] has reported that treatment with pefloxacin is associated with increased activities of ALT and AST. Similarly, artequin® had earlier been reported to be safe for the treatment of uncomplicated malaria with non-significant increases in serum enzyme activities [20][21].The results of the present study is in line with other reports in the literature [22][19][23]. The kidneys are the major organs involved in the maintenance of electrolyte balance and fluid volume [24]. The functional integrity of the kidney can be evaluated by assessing biomarkers such as blood urea, creatinine and electrolytes (e.g. sodium, potassium, chloride, bicarbonate) [25]. Creatinine is produced from creatine phosphate in muscles. Synthesis of creatinine takes place at a constant rate. It is filtered out of blood by the kidneys. Blood level of creatinine is usually elevated in situations where the filtering capacity of the kidney is defective [25]. The significant elevation of serum creatinine observed in this study is an indication of renal dysfunction induced by drug administration. Flouroquinolone antibiotics have been listed as a group of drugs that induce renal toxicity [26]. Serum urea is another useful indicator of renal function [27]. Urea is a major end product of protein metabolism produced in the liver and secreted in kidneys [28]. Serum concentration of urea is observed to rise equally when renal function is compromised [25]. In the present study, serum urea was observed to be significantly high ($p<0.05$) as a consequence of co-administration with artequin® and pefloxacin. Sodium is the principal cation in extracellular fluid in which the physiological

concentration is regulated by the kidneys [25]. The present study did not reveal any significant changes in circulating levels of sodium ions. It has been reported that sodium balance is usually maintained even in disturbances that cause major changes in kidney function [27]. Potassium is the major cation in the extracellular fluid. The maintenance of potassium balance is dependent on excretion by the kidneys [27]. Fluctuation in serum potassium levels has serious health implications [29]. Hypokalaemia can result in muscular weakness and cardiac arrhythmia whereas hyperkalaemia is a risk factor for cardiac arrest [25]. In the present study, drug administration caused a significant decrease ($p<0.05$) in serum potassium ion levels. This can be attributed to renal dysfunction [30]. Bicarbonate and chloride ions in serum are also used to assess kidney function. The kidneys participate in the regulation of acid base balance primarily by the conservation of bicarbonate ions [28]. Therefore, the significant increase in serum bicarbonate and chloride ions observed in this study indicates loss of functional capacity of the kidneys [31]. Histopathological evaluation of the liver revealed areas of vascular congestion, vascular/ cellular degeneration and pyknotic nuclei which corroborated with the changes in serum enzyme activities. DILI is generally classified as direct and indirect toxicity. Direct toxicity includes injuries caused directly by the xenobiotic or its metabolites as is the case with acetaminophen [32]. Pefloxacin is particularly metabolised to norfloxacin, a more active metabolite by the liver CYP450 enzyme system [33] which also leads to production of reactive oxygen species and a reduction in glutathione (GSH), hence the observed toxicity. Indirect toxicity is a more complicated and less understood process. It involves inflammatory process, including activation of innate and or adaptive immune response [34]. Minor hepatocellular dysfunction and cell death caused by therapeutic drugs or other factors may trigger the activation of cells involved in the innate immune system such as kupfer cells (resident macrophages of the liver) and natural killer cells. These may then exacerbate initial minor injury by activating the adaptive immune response system producing pro-inflammatory cells to the liver [35]. Similarly, histology of the kidneys demonstrated glomerular inflammation, cellular distortion and pyknotic nuclei as a consequence of drug administration which is in line with results obtained for serum creatinine and urea. The present study has shown that co-administration of pefloxacin and artequin® increases the risk of development of hepatocellular and renal injury. Although the present study did not examine the possibility of recovery after treatment, there is need for regular monitoring of liver and kidney functions in the course of administration with artequin® and pefloxacin especially in situations of repeated or long term treatment.

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Table 1. Effect of Co-Administration of Therapeutic Doses of Pefloxacin and Artequin® on Serum Enzyme Activity in Mature Male Albino Wistar Rats.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
A	47.00 ± 1.22	154.25 ± 1.38	85.20 ± 0.19
B	49.00 ± 0.89	177.20 ± 0.97[a]	112.23 ± 0.10[a]
C	49.02 ± 0.81	17.20 ± 0.80[a,b]	110.23 ± 0.07[a]
D	52.20 ± 0.80[a,b,c]	177.00 ± 0.55[a,c]	118.44 ± 0.25[a,b,c]

Table 2. Effect of Co-Administration of Therapeutic Doses Pefloxacin and Artequin® on Indices of Renal Function in Mature Male Albino Wistar Rats.

Groups	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO^- (mmol/L)	Creatinine (µmol/L)	Urea (mmol/L)
A	140.25 ± 1.26	5.83 ± 0.43	103.5.0 ± 0.50	27.50 ± 0.68	86.65 ± 0.52[d]	4.76 ± 0.19
B	139.60 ± 1.14	4.36 ± 0.40[a]	103.00 ± 0.95	28.70 ± 0.80	97.34 ± 0.70[a]	4.87 ± 0.10
C	140.60 ± 1.14	5.22 ± 0.18[a,b]	110.80 ± 2.85[a,b]	32.90 ± 0.60[a,b]	96.58 ± 1.06[a]	5.02 ± 0.07
D	139.60 ± 1.14	5.00 ± 0.12[a,b]	108.60 ± 0.75[a,b]	28.70 ± 0.49[c]	103.56 ± 0.38[a,b,c]	5.87 ± 0.25[a,b,c]

Values are expressed as Mean ± Standard Error of Mean.
a = significantly different from Group A ($p<0.05$);
b = significantly different from Group B ($p<0.05$);
c = significantly different from Group C ($p<0.05$).

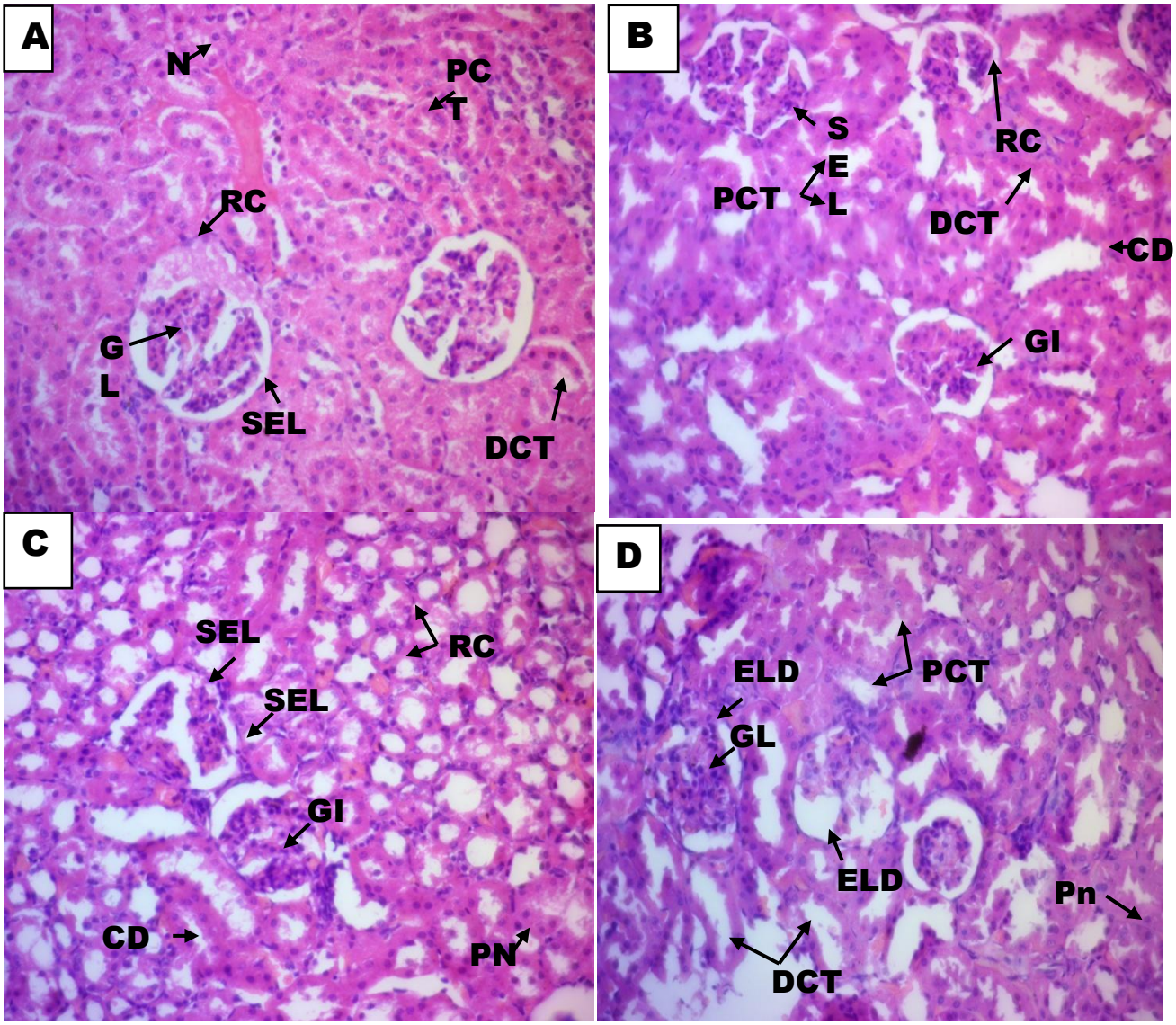

Figure 1. Photomicrographs of kidney at magnification x400 (A) control; (B) administered 6.67 mg of pefloxacin per kg bw twice daily; (C) administered artequin® (5 mg of mefloquin and 4 mg of artesunate per kg bw daily); (D) administered pefloxacin + artequin® as in (B) and (C).

Key: Renal corpuscle (RC) Distal convoluted tubules (DCT), Proximal convoluted tubules (PCT), Collecting ducts (CD), Squamous epithelial lining (SEL), Glomerular Inflammation (GI), Epithelial lining degeneration (ELD), Tubular Degeneration (TD), Cellular degeneration (Cd) Pyknotic nucleus (Pn) and Nucleus (N)

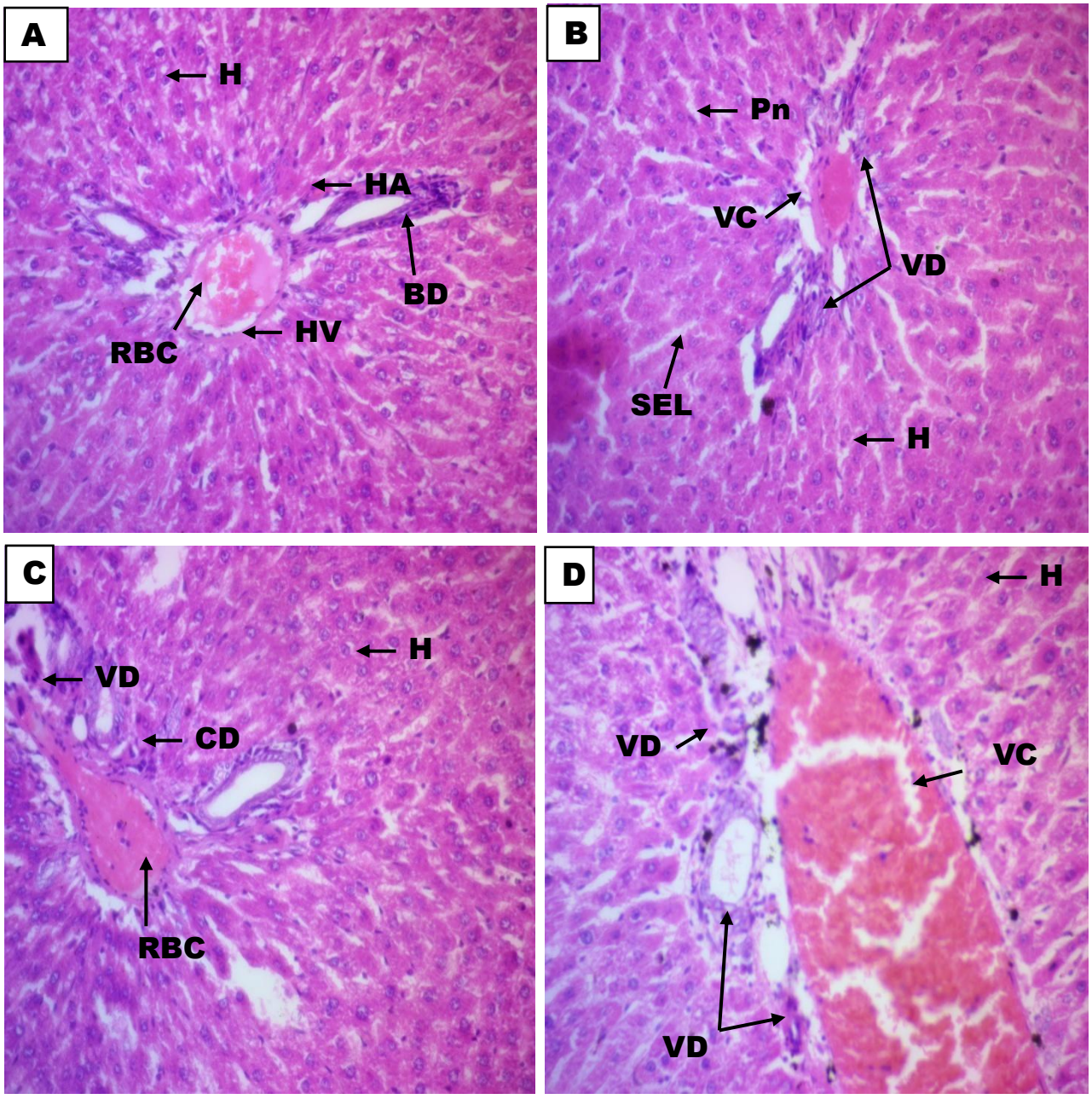

Figure 2. Photomicrographs of liver at magnification x400 (A) control; (B) administered 6.67 mg of pefloxacin per kg bw twice daily; (C) administered artequin® (5 mg of mefloquin and 4 mg of artesunate per kg bw daily); (D) administered pefloxacin + artequin® as in (B) and (C).

Key: Portal triade (PT), Bile Duct (BD), Hepatic Artery (HA), Hepatic Vein (HV), Hepatocytes (H), Nucleus (N), Vascular Congestion (VC), Cellular Degeneration (CD), Vascular Degeneration (VD), Pyknotic Nucleus (Pn) and Focal Area of Inflammation (I), Squamous Epithelial Lining (SEL), Red Blood Cell (RBC)