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Research Article

Oral Acute Toxicity of Acetaminophen in Nigerian Local Dogs

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Abstract

Acetaminophen is the most commonly used analgesic and antipyretic agent throughout the world. In Nigeria with large dog population it is used to control pain in veterinary clinics. This study is aimed at determining the acute toxicity of acetaminophen in Nigerian local dogs. Three dogs were used for the study using the up and down method. Blood samples were collected prior to acetaminophen administration orally and at day 14 post-administration. The blood was used for haematology and serum biochemistry. Post mortem was performed on the dead dog and tissue samples obtained for histo-pathological examination. The dog treated with acetaminophen (1000 mg/kg) died within 24 ± 3.5 hours, while those treated with 500 mg/kg survived. The oral median lethal dose (LD_{50}) was calculated to be 629.96- mg/kg indicating moderate toxicity. The haematological (RBC, PVC and Hb) values obtained pre-treatment were higher than those obtained post-treatment. Elevated biochemical (liver enzymes, BUN and creatinine) values were obtained post-treatment compared to the values obtained pre-treatment. Histopathological lesions occurred in the liver, kidney and intestine of the dead dog. The presence of anaemia, elevated liver enzymes, increased BUN and creatinine, and lesions in organs and tissues is suggestive of generalized toxicity.

Keywords: Toxicity; Acetaminophen; Dog; Haematology; Histology

Introduction

Acetaminophen is the most common cause of acute liver failure in man in many western countries [1,2]. It is one of the most commonly used over the counter antipyretic and analgesic agent worldwide [3].

Acetaminophen has several complex pathways including the peripheral (COX inhibition), and central (COX serotonergic descending neuronal pathway, L arginine/NO pathway, cannabinoid system), antinociception process and "redox" mechanism [4].

The metabolism of acetaminophen occurs in the liver, where it undergoes biotransformation by glucoronic acid conjugation and sulphur oxidation and is excreted by the kidney [5-7]. Acetaminophen toxicity results from over dose of the drug [8]. The toxicity due to both intentional and non-intentional over dose of this drug has affected humans and animals for decades and is as a result of interference with the metabolic pathway that involves the microsomes within the hepatocytes [9]. In Nigeria, with large number of dog population, there is no available research work on the toxicity of acetaminophen in dogs, although the drug is used regularly for

the treatment of pain in small animals in the veterinary hospitals and clinics. The objective of this study is to determine the acute toxicity of acetaminophen in dogs.

Materials and Methods

Experimental animals and treatments

Three Nigerian local breed of dogs of twelve months of age, obtained within Makurdi, Benue state, of Nigeria were used. They were housed separately in the dog kennels at the Veterinary Teaching Hospital, University of Agriculture, Makurdi, Benue state, Nigeria. They were fed with dog food and clean water and were screened for the presence of haemoparasites and de-wormed with ivermectin at the dose of 0.4 mg/kg subcutaneously. The animals were acclimatized for two weeks for the study.

Acetaminophen (1000 mg/kg) was administered orally to one dog and observed for 48 hr for signs of toxicity and death [10]. Since the dog died within 24 hr, a second dog was given a decreased dose (500 mg/kg) and observed for 48 hr and thereafter for 14 days for delayed toxicity. Because the dog survived, the third dog was given the same dose and observed for the same period for toxicity signs. The median lethal dose (LD_{50}) was thereafter calculated making use of geometric mean [11].

Blood samples were obtained from the dogs before drug administration and at 14 days post drug administration. At sampling periods two blood samples were collected, one with anti-coagulant [ethylene diamine tetrat-acetic acid (EDTA)], and the other without anti-coagulant. Hematologic parameters (red blood cells count (RBC), haemoglobin concentration (Hb), packed cell volume (PCV), total white blood cells count (WBC) and differential leucocytes count) were determined with blood samples with anti-coagulant. Biochemical parameters (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatise (ALP), total protein (TB), blood urea nitrogen (BUN) and creatinine) were determined with blood without anticoagulant. Tissue samples of the liver; kidney and intestine from the dead dog were collected and processed for examination of histopathological lesions.

Hematology assay

Hematological parameters were analysed with automatic hematology analyser (Shenzhen Mindray Bio-Medical Electronic Co. LTD. China).

Biochemical assay

Serum level of ALT and AST were determined using the method of Reitman and Frankel [12], and serum ALP level was assayed by Babson and others [13] procedure. Serum total protein level was determined by the method of Tietz [14], while creatinine level was assayed by Jaffe's reaction as described by Bishop [15] and BUN by Fawcett and Scott [16] method. Randox® commercial kits (Randox Lab. LTD. UK) were used for the various assays.

Histopathology

Tissue samples from the dead dog were fixed in 10% formal-saline for a minimum of 24hr then dehydrated by washing in ascending grades of ethanol before clearing with xylene and embedding in paraffin wax. The samples were sectioned with microtone, stained with Hematoxylin and Eosin (H and E) and mounted on Canada balsam [17]. All sections were examined under light microscope magnifications. Photographs of lesions were taken with an Olympus photomicroscope for observation and comparison of histopathologic lesions.

Statistical analysis:

All values obtained were expressed as the average of two observations. The difference between the pre-treatment and post-treatment values were expressed in percent [18].

Results

The dog administered acetaminophen orally at the dose of 1000 mg/kg body weight died within 24 hr of the drug administration. The two dogs treated with 500 mg/kg acetaminophen survived beyond 14 days without any visible signs of toxicity. The calculated oral LD50 for acetaminophen in Nigerian local dog was 629.96 mg/kg.

The effect of 500 mg/kg acetaminophen on haematological parameters is presented in table 1. The pre-treatment values for RBC count, Hb concentration and PCV values were higher than those obtained post-treatment. The pre-treatment value for total WBC count was lower than that obtained post-treatment. The post-treatment lymphocyte value was higher than the pre-treatment value. The other haematological parameters were not seriously altered by acetaminophen treatment.

Acetaminophen (500 mg/kg) increased the biochemical parameters of the treated dogs. The post-treatment values when compared to the pre-treatment values were higher except for TP in

which the pre-treatment value was slightly higher compared to the post-treatment value (Table 2).

Histopathological changes occurred in the liver, kidney and intestine of the dog treated with 1000 mg/kg acetaminophen. The liver showed a deviation from the normal hepatic histo-architecture. There was diffused hepatocellular necrosis and infiltration of inflammatory cells (Figure 1). The kidney showed hypoplastic glomerulus and nephritic tubules (Figure 2). In the intestine, there was necrosis with infiltration by mononuclear inflammatory cells. There was also sloughing of epithelium of the intestine into the lumen, and sub-epithelia oedema (Figure 3).

Numbers of groups	Dosage of Silybon 140® (mg/kg)	Numbers of Dogs	Numbers of Death	% Mortality
1	1000	1	1	100
2	500	1	0	0
3	500	1	0	0

Table 1: Results of Acute Oral Toxicity of Acetaminophen and LD50 Determination.

LD_{50} = (1000 x 500 x 500) 1/3 = 629.96 mg/kg.

Parameter	Value	
	Pre-treatment	Post-treatment
PCV (%)	42.10 ± 2.56	29.80[a]
Hb (g/L)	120.60 ± 13.01	64.18[a]
RBC (%)	5.78 ± 0.64	3.95[a]
WBC (%)	14.84 ± 1.44	18.22[b]
NEUT (%)	10.20 ± 2.12	9.49[a]
LYMP (%)	3.70 ± 3.63	7.40[b]
MONO (%)	0.73 ± 0.84	0.81
EOSI (%)	0.61 ± 0.05	0.77
BASO (%)	0.0074 ± 0.01	0.00

Table 2: Effect of acetaminophen (500 mg/kg) administration on mean values of haematological parameters during acute oral toxicity studies in dogs.

a = significantly decreased ($P < 0.05$) compared to pre-treatment values.

b = significantly increased ($P < 0.05$) compared to pre-treatment values.

Parameter	Value	
	Pre-treatment	Post-treatment
ALT (IU/L)	11.36 ± 1.01	40.54[a]
AST (IU/L)	26.86 ± 3.15	80.92[a]
ALP (IU/L)	220.11 ± 5.45	332.53[a]
TP (g/L)	76.52 ± 2.14	72.67
Urea (g/L)	20.55 ± 1.22	120.32[a]
Creatinine (g/L)	2.34 ± 0.51	8.86[a]

Table 3: Effect of acetaminophen (500 mg/kg) administration on Mean value of some biochemical parameters during the acute oral toxicity studies in dogs.

* = Average value of some biochemical parameters.

a = Significantly increased ($P < 0.05$) compared to pre-treatment values.

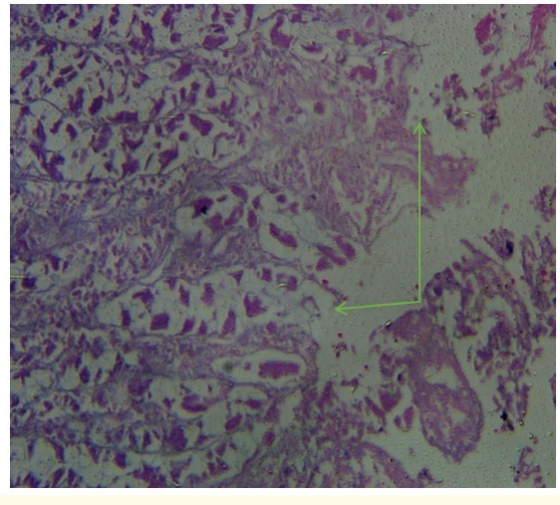

Figure 1: Section of intestinal mucosa of dog administered acetaminophen in acute oral toxicity study showing sloughing of epithelium (arrow) H and E (x 10).

Discussion

Acetaminophen in the present study caused mortality in dogs at the dose of 1000 mg/kg, with an oral LD_{50} of 629.96 mg/kg. This is an indication that the drug is moderately toxic, since its LD_{50} lies between 500 and 1000 mg/kg body weight [19].

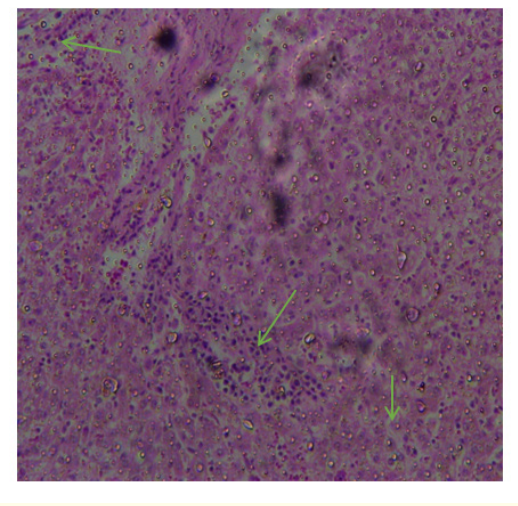

Figure 2: Liver of dogs administered acetaminophen in acute oral toxicity study showing multifocal areas of hepatocellular distruction (arrow) accompanied by massive infiltration by mononuclear cells made up largely of lymphocytes. H and E (x 10).

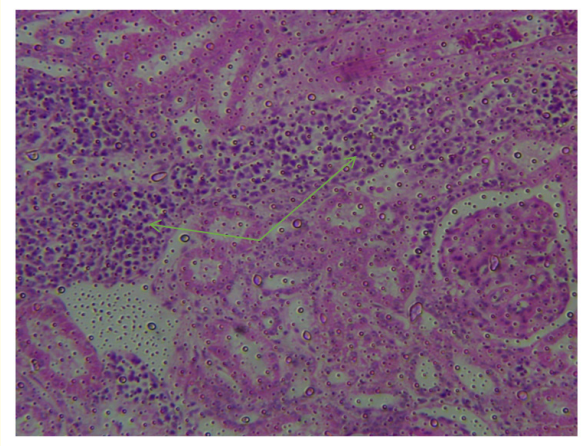

Figure 3: Kidney of dogs administered acetaminophen in acute oral toxicity study showing a focus of massive interstitial nephritis accompanied by marked infiltration by mononuclear cells (arrow). H and E (x 10).

The haematological parameters of dogs treated orally with 500 mg/kg of acetaminophen were significantly altered when the pre-treatment and post-treatment values were compared. The observed decrease in haematological parameters (RBC, Hb, and PCV) may indicate anaemia. The anaemia could have occurred as a result of hemolysis or inhibition of hematopeisis in the bone marrow [20]. Treatment with acetaminophen also changed the WBC count significantly ($P < 0.05$). The increase in WBC count was due to lymphocytosis (Table 2).

The elevation of hepatic enzymes observed in the present study as a result of acetaminophen administration may be indicative of liver injury and this may have caused leakage of cellular enzymes (ALT, AST and ALP) into the serum. When the plasma membrane of hepatocytes is damaged, various enzymes which normally are present in cytosol are released into the blood stream [21]. The post-treatment value of serum total protein of dogs treated with acetaminophen was slightly reduced, and this may suggest that the intestinal lesions may not be serious enough to prevent absorption of nutrients. The observed elevation in serum urea and creatinine level post-treatment with acetaminophen may indicate kidney problem. Blood urea nitrogen test is a measure of the amount of nitrogen in bloods that comes from urea. It is used as a marker for renal function, though; it is inferior to other markers including that of creatinine because urea level could be influenced by other factors such as diet and dehydration [22]. Creatinine is a waste product of creatine which is present in skeletal muscle as creatine phosphate a high energy compound. Serum creatinine determination is one of the means of testing for renal function, reflecting the balance between its production and filtration by renal glomerulus [23]. Congestion of organs and tissues of the dead dog treated with acetaminophen (1000 mg/kg) were observed. The generalized focal areas of necrosis with mononuclear cellular infiltration observed in the tissues were indicative of cellular death and the body's attempt to remove the necrotic debris at these areas. The presence of lesions in the liver may be due to the fact that the liver is the primary organ of bio-transformation of most chemical agents including acetaminophen [24,25].

The kidney was also adversely affected by 1000 mg/kg dose of acetaminophen. The necrotic degenerative changes observed in tubular epithelium with attendant mononuclear cellular infiltration suggest that the toxic intermediate may be excreted through the kidney and is toxic to tubular epithelial calls.

Conclusion

In conclusion therefore, the oral median lethal dose of acetaminophen in Nigerian local dogs was found to be 629.96 mg/kg, indicating a moderate margin of safety. The presence of anaemia increased liver enzymes, elevated level of BUN and creatinine and lesions in organs and tissues is suggestive of generalised toxicity, and hence the drug should be used with caution in this specie of animal.

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Conflict of Interest

The authors hereby declare that there is no conflict of interest regarding this work.

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