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ORIGINAL ARTICLE

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Hepatoprotective Effect of Silybon 140® on Acetaminophen induced Liver Toxicity in Nigerian Local Dogs

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Abstract
Acetaminophen is commonly used in veterinary practice and it is known to produce hepatotoxicity at high doses especially in companion animals. Silybon 140® (*Silybum marianum*) is a new drug for the treatment of several liver injuries in humans. This experiment investigated the protective effect of Silybon 140 on toxicities induced by acetaminophen in dogs. Twelve Nigerian dogs about 12 months of age were randomly separated into four groups of three dogs each. Group 1 received only distilled water throughout the experiment while groups 2 and 3 were administered Silybon 140 at dose of 100 and 200 mg/kg for three days respectively. A day after, groups 2, 3 and 4 were administered acetaminophen at dose of 500 mg/kg once. All medications were given orally. Blood samples were collected in two samples bottles; one with EDTA and the other without EDTA for hematology and serum biochemistry studies. Blood samples were collected before drug administration and at day 7, 14 and 21 post acetaminophen administration. The results showed significant protective effect of Silybon 140 on the liver and kidney against acetaminophen toxicity. At day seven there was significant decrease in AST levels in Silybon treated groups and ALT levels at day 21 comparing to acetaminophen group. Similarly, creatinine levels of 1.7±0.1, 1.6±0.3 and 2.7 ±0.3 for groups 2, 3 and 4 respectively were observed. At the doses of 100 and 200 mg/kg, Silybon 140 effectively protected the liver and kidney against acetaminophen induced renal and hepatic toxicity, thus can be used for disease conditions affecting the liver and the kidney in dogs.

Keywords
Acetaminophen, Dog, Silybon 140®, Toxicity

1. Introduction
The liver is the largest visceral organ in the body **(Taylor et al., 2005)** and the major metabolic organ of the animal's body **(Kelly et al., 1992)** with critical roles such as detoxification of metabolic wastes, destruction of spent red blood cells and reclamation of their constituents, and contributing for adequate function of other organs **(Invernizzi, 2013; Chatterjee and Mitra, 2015; Raschzok et al., 2015).** The liver is however susceptible to toxic injuries directly or indirectly as a result of exposure to foreign substances or due to increased exposure to high concentration of ingested compounds or their metabolites. Generation of potentially toxic metabolites from an otherwise harmless parent compound is the mechanism believed to be responsible for most hepatic injuries caused by therapeutic agents **(Craig and Stize, 1994; Farrel and Liddle G 2002).** Acetaminophen also known as APAP (in the United State), Paracetamol® (in Europe and other area of the world) or N-acetyl-p-aminophenol is one of the most commonly used over the counter antipyretic and analgesic agent worldwide **(Bunchorntavakul and Reddy, 2013).** Acetaminophen is the most common cause of acute liver failure in many western countries **(Budnitz et al., 2011; Manthripogada et al., 2011).** It is used in the experimental induction of acute liver injury in animals. The increasing prevalence of liver diseases in dogs underscores the need to search for more effective and cost effective treatments. The extract of *Silybum marianum*, a plant belonging to family *Carduus maranum* **(Pradhan and**

Girish, 2006) which has scaled clinical trials is today the leading drug in the management of hepatic injury (Kren and Walterova, 2005; Gholamreza et al., 2011). Silymarin has a regulatory action on cellular and mitochondrial membrane permeability and therefore inducing membrane stability against xenobiotics injury (Muriter et al., 1986). It can prevent the absorption of toxins into the hepatocytes by occupying the binding site as well as inhibiting many transport proteins at the membrane (Faulstich et al., 1980) and by inhibiting the effect on the cytochrome p450 enzyme (Baer–Duhowska et al., 1998).

2. Materials and Methods

2.1 Experimental Drugs

Silybon 140® (Silymarin, Micro. Labs, Ltd, H.P. India) and Acetaminophen (Paracetamol®, Pharmetex Pharmaceutical Industry LTD, Lagos, Nigeria) were obtained from the Veterinary Teaching Hospital, Federal University of Agriculture, Makurdi.

2.2 Animals and Treatments

Twelve dogs of 12 months old were used for this study. The dogs were maintained in standard dog kennels with a temperature (31±3°C) and 40% humidity. The dogs were fed on standard dog feed with free access to clean drinking water. The animals were acclimatized for 2 weeks before the commencement of the experiment. The study was conducted on approval of the College of Veterinary Medicine, Federal University of Agriculture Ethics Committee. The dogs were separated into four groups, 1–4, of three dogs each. Group 1 served as the control and was administered only distilled water. Groups 2 and 3 were given Silybon 140® at the rate of 100 and 200 mg/kg body weight respectively for 3 days after which acetaminophen was then administered once to both groups 2, 3 and 4 at the dose of 500 mg/kg orally. Blood samples were obtained from the dogs before the drug treatments and thereafter, on days 7, 14 and 21, 0.5mls blood samples each were obtained at each sampling period from each dog, one without anti-coagulant and the other with anti-coagulant (EDTA). The blood samples with anti-coagulant were used for hematological parameters determination, while the blood samples without anti-coagulant were used for the determination of biochemical parameters. The hematological and biochemical parameters were determined as stated earlier above.

2.3. Histopathological Studies

Tissue samples were collected from the liver and kidneys of all animals. These samples were immediately fixed in formalin 10% for at least 48hrs, and then, washed, dehydrated, cleared and embedded in paraffin wax. 4-5 micron tissue sections were mounted on glass slides and stained with H&E stain according to Suvarna et al., (2019).

2.4. Statistical Analysis

Data obtained from this study were expressed as mean value ± standard error of mean (S.E.M.). Vital parameter data were compared by student t-test. Two Way Analysis of variance was used for all the parameters using Graph Pad Prism software. A probability of less than 5% ($P<0.05$) was considered significant.

3. Results

3.1. Effects of Pre-treatment with Silybon 140® on Hematological Changes Induced by Acetaminophen Administered in Dogs

The mean packed cell volume (PCV) of dogs treated with Silybon 140®, followed by acetaminophen is presented in Table (1). The mean PCV of the control group ranged from 50.0 ±5.7 to 51.7 ± 5.2%. However the PCV values of the ACE-only treated group ranged from 30.4±3.7% to 40.1±2.6% over the 21 day post treatment observation period which were significantly lower the values of the control group. However the groups with the prior exposure to 100mg/kg or 200mg/kg Silybon 140® and then later administered ACE behaved alike resulting to PCV values very close to those of the control dogs. The mean RBC counts of dogs given different doses of Silybon 140® followed by acetaminophen are presented in Table 2. The variations in the RBC counts of the control, ACE-only group and the groups treated with either 100 m/kg or 200mg/kg of Silybon 140® plus acetaminophen closely followed the pattern of changes in PCV. The mean hemoglobin values of dogs treated with Silybon 140 and later administered acetaminophen is summarized in Table 3. The hemoglobin (Hb) concentration of control dogs ranged from 156.0 ± 12.8 till 162.7 ±11.6 g/L. However the mean Hb value of dogs administered only acetaminophen decreased significantly ($P<0.05$) when compared to that of the control throughout the 21 day post treatment evaluation period. The groups pre-treated with 100mg/kg or 200mg/kg Silybon 140® for 3 days and thereafter treated with 500mg/kg of acetaminophen had significantly ($P<0.05$) improved Hb concentrations which were higher than those of the ACE-only treated dogs over the 21 day observation period. However these increases did not attain to those of the control dogs. The mean White Blood Cell count of control dogs varied between 16.3 ± 0.7 to 14.3 ± 2.1 (x 10^9/L) during the period of this study (Table 4). However the ACE-only treated group showed drop in WBC values that varied between 9.5± 0.7 and 12.1± 0.8 (x 10^9/L) within the 21 day post treatment observation period. The groups treated with Silybon 140® at either 100mg/kg or 200 mg/kg followed later by acetaminophen had significantly ($P<0.05$) higher WBC counts when compared to those of ACE-only treated group, the values being closer to those of control.

Table 1. Mean ±SEM Pack Cell Volume of Dogs pre-treated with Silybon 140® prior to 500mg/kg Acetaminophen administration.

Treatment Groups	Packed Cell Volume (%) Days of Sampling			
	0	7	14	21
Group 1	51.7±5.2	51.7±4.8	50.0±5.7	51.6±4.2
Group 2	44.6±5.8	44.6±4.3	46.8±2.4	48.3±3.6
Group 3	39.7±4.9	43.4±4.5	49.6±4.8	50.3±5.6
Group 4	34.4±4.3	30.4±3.7[a]	32.4±4.1[a]	40.1±2.6[a, b]

Group 1 = Control, distilled water administered dog, Group 2 = Silybon 140® 100 mg/kg pre-treated dogs followed by acetaminophen administration. Group 3 = Silybon 140® 200 mg/kg pre-treated dogs followed by acetaminophen administration. Group 4 = acetaminophen only administered group. Significantly decrease along column a; significantly increase along column b

Table 2. Mean±SEM Red Blood Cell Count of dogs pre-treated with Silybon 140® pre-treatment prior to 500mg/kg acetaminophen administration.

Treatment Groups	Red Blood Cells Count ($x10^{12}$/L) Days of Sampling			
	0	7	14	21
Group 1	7.6±0.5	7.6±0.3	7.1±0.2	7.6±0.4
Group 2	7.0±0.6	7.0±0.6	7.1±0.4	7.4±0.2
Group 3	6.8±0.1	6.9±0.1	7.4±0.3[d]	7.5±0.4[d]
Group 4	6.2±0.2	5.1±0.3[ac]	5.5±0.1[ac]	6.3±0.4[a]

Group 1 = Control, distilled water administered dog. Group 2 = Silybon 140® 100 mg/kg pre-treated dogs followed by acetaminophen administration. Group 3 = Silybon 140® 200 mg/kg pre-treated dogs followed by acetaminophen administration. Group 4 = acetaminophen only administered group. Significantly decrease along column a; significantly decrease along row c; significantly increase along column b; significantly increase along column d

Table 3. Mean±SEM Hemoglobin Concentration of Dogs pre-treated with Silybon 140® prior to 500 mg/kg acetaminophen administration.

Treatment Groups	Hemoglobin Concentration (g/L) Days of Sampling			
	0	7	14	21
Group 1	162.7±11.6	162.7±13.7	156.0±12.8	162.7±13.3
Group 2	137.3±18.4	154.0±16.2	147.3±14.6	138.7±13.3
Group 3	136.7±13.3	129.3±14.7[a]	143.0±42.5	146.8±14.5
Group 4	149.0±10.9	90.0 ±8.6[ac]	106.3±10.4[ac]	119.3±11.8[ac]

Group 1 = Control, distilled water administered dog. Group 2 = Silybon 140® 100 mg/kg pre-treated dogs followed by acetaminophen administration. Group 3 = Silybon 140® 200 mg/kg pre-treated dogs followed by acetaminophen administration. Group 4 = acetaminophen only administered group. Significantly decrease along column a; significantly decrease along row c; significantly increase along column b; significantly increase along column d

Table 4. Mean ± SEM White Blood Cell Count of dogs pre-treated with Silybon 140®prior to acetaminophen administration.

Treatment Groups	White Blood Cells Count ($x10^9$/L) Days of Sampling			
	0	7	14	21
Group 1	16.3±0.7	16.3±2.2	15.9±1.8	14.3±2.1
Group 2	14.7±2.4	21.2±0.9[b,d]	17.5±2.8	12.2±0.7
Group 3	12.1±0.2	14.1±3.7	16.8±3.0	14.2±0.9
Group 4	11.1±0.6	12.1±0.8	11.6±1.1[a]	9.5±0.7[a,c]

Group 1 = Control, distilled water administered dog. Group 2 = Silybon 140® 100 mg/kg pre-treated dogs followed by acetaminophen administration. Group 3 = Silybon 140® 200 mg/kg pre-treated dogs followed by acetaminophen administration. Group 4 = acetaminophen only administered group. Significantly decrease along column a; significantly decrease along row c; significantly increase along column b; significantly increase along column d

3.2. Effects of Pre-treated with Silybon 140®on the Serum Biochemistry of Acetaminophen Treated Dogs

Increase in serum aspartate aminotransferase (AST) activity was observed in the group treated with acetaminophen alone **(Table, 5).** The increase was significant (P<0.05) when compared to the control throughout the period of study. In the groups treated with silybon 140®at the dose of either 100mg/kg or 200mg/kg, behaved the same,the only noticeable increase being Serum alanine aminotransferase (ALT) in the group treated with only acetaminophen was also significantly (P<0.05) increased from day 7 to 21, when compared to the control and the pre-treatment values.
on day 7 and the increases were not as high as that of acetaminophen group. The AST value of the control dogs varied within normal range throughout the study period. The groups administered 100mg/kg silybon 140®plus acetaminophen and 200mg/kg silybon 140® plus acetaminophen had significantly (P< 0.05) increased ALT values at day 7 only.

The ALT values for the control group were similar throughout the period of the study **(Table, 6).** The alkaline phosphatase activities in dogs treated with Silybon 140® and thereafter given acetaminophen **(Table, 7)**. The group treated with acetaminophen only had significantly ($P<0.05$) increased values throughout the period of the study. The groups treated with either 100mg/kg or 200mg/kg of Silybon 140® before acetaminophen administration had alkaline phosphatase activity increase only on day 7. The activity of this enzyme in the control group was not altered throughout the study period.

The total protein concentrations **(Table, 8)** in the serum of treated animals appear to be comparable to that of the control group except those of dogs treated with acetaminophen alone, which was higher than those of the other treatment groups from days 7 to 21. The effect of the acetaminophen on serum urea level showed that there was an increase in serum urea concentration of animals treated with acetaminophen alone compared with the control **(Table, 9).** Treatment with Silybon 140® appear to decrease the serum urea levels. The animals administered only acetaminophen had a significantly ($P<0.05$) increased urea concentration of 28.6 ± 1.6mg/dl compared to that of the control (11.0 ±0.8mg/dl) at 7 day of treatment. The urea levels of the group treated with silybon 140® were on day 7 not as high as that of the group treated with acetaminophen alone, although they were higher than that of the control group. Also Silybon 140® treatments decreased significantly ($P<0.05$) the urea levels of the treated animal below those of the control group and the group administered acetaminophen alone on days 14 and 21. Creatinine concentrations also decreased following treatments with Silybon 140® in animals administered acetaminophen **(Table, 10).** The animals treated only with acetaminophen showed increased creatinine concentrations from days 7 to 21, when compared with the control group and those treated with acetaminophen and Silybon 140® combinations. The total bilirubin concentrations of dogs given Silybon 140® plus acetaminophen is presented in **Table (11).** Following treatment with acetaminophen alone, total bilirubin concentrations increased significantly ($P<0.05$) when compared to the control, and acetaminophen plus Silybon 140® combination groups. The increase in bilirubin was noticeable throughout the study period in the group given acetaminophen alone.

Table 5. Mean ± AST activity of dogs pre-treated with Silybon 140® prior to acetaminophen administration.

Treatment Groups	AST(IU/L) Days of Sampling			
	0	7	14	21
Group 1	20.0±0.8	19.0±3.4	20.0±1.8	20.8±0.9
Group 2	19.3±1.5	30.7±9.7[a]	18.0±0.6	18.0±0.6
Group 3	19.0±1.5	26.0±4.1[a]	17.3±2.2	16.0±2.1
Group 4	20.0±2.1	97.3±6.7[a,b]	86.7±13.8[a,b]	83.0±14.5[a,b]

Group 1 = Control, distilled water administered dog. Group 2 = Silybon 140® 100 mg/kg pre-treated dogs followed by acetaminophen administration.
Group 3 = Silybon 140® 200 mg/kg pre-treated dogs followed by acetaminophen administration. Group 4 = acetaminophen only administered group.
Significantly decrease along column a; significantly decrease along row

Table 6. Mean ± SEM ALT activity in dogs pre-treated with Silybon 140® prior to acetaminophen administration.

Treatment Groups	ALT(IU/L) Days of Sampling			
	0	7	14	21
Group 1	13.0±1.6	13.7±1.2	13.7±1.6	13.0±0.8
Group 2	13.3±1.5	30.0±2.5[b,d]	14.7±1.9	13.7±1.8
Group 3	12.7±1.5	25.9±4.7[b,d]	12.0±2.6	8.3±0.9[a,c]
Group 4	13.0±1.4	14.2±5.6	47.3±6.8[b,d]	49.3±8.4[b,d]

Group 1 = Control, distilled water administered dog. Group 2 = Silybon 140® 100 mg/kg pre-treated dogs followed by acetaminophen administration.
Group 3 = Silybon 140® 200 mg/kg pre-treated dogs followed by acetaminophen administration. Group 4 = acetaminophen only administered group.
Significantly decrease along column a; significantly decrease along row c; significantly increase along column b; significantly increase along column d

Table 7. Mean ± SEM Alkaline Phosphatase activity of dogs pre-treated with Silybon 140® prior to acetaminophen administration.

Treatment Groups	Alkaline Phosphatase (IU/L) Days of Sampling			
	0	7	14	21
Group 1	30.3±3.1	28.7±2.7	29.0±1.3	29.0±1.5
Group 2	29.7±2.4	34.0±3.2	27.7±1.5	23.7±3.2[a]
Group 3	30.7±2.6	37.7±3.8[b]	31.0±0.6	29.0±0.6
Group 4	28.7±2.4	82.3±8.6[b,d]	79.3±7.6[b,d]	81.0±9.2[b,d]

Group 1 = Control, distilled water administered dog. Group 2 = Silybon 140® 100 mg/kg pre-treated dogs followed by acetaminophen administration.
Group 3 = Silybon 140® 200 mg/kg pre-treated dogs followed by acetaminophen administration. Group 4 = acetaminophen only administered group.
Significantly decrease along column a; significantly decrease along row c; significantly increase along column b; significantly increase along column d

Table 8. Mean ± SEM Total protein concentration of dogs pre-treated with Silybon 140® prior to acetaminophen administration.

Treatment Groups	Total Protein (g/L) Days of Sampling			
	0	7	14	21
Group 1	48.7±0.7	47.0±1.1	49.0±1.3	49.7±0.8
Group 2	48.0±1.2	54.0±1.2^{bd}	50.0±0.6	51.0±0.6
Group 3	49.0±4.4	54.3±0.8^{b}	51.3±0.9	50.3±0.3
Group 4	48.3±3.1	64.3^{a}±5.7^{bd}	59.0±3.0^{b,d}	57.3±0.8^{b,d}

Group 1 = Control, distilled water administered dog. Group 2 = Silybon 140® 100 mg/kg pre-treated dogs followed by acetaminophen administration. Group 3 = Silybon 140® 200 mg/kg pre-treated dogs followed by acetaminophen administration. Group 4 = acetaminophen only administered group. Significantly decrease along column a; significantly decrease along row c; significantly increase along column b; significantly increase along column d

Table 9. Mean± SEM Urea Concentration of dogs pre-treated with Silybon 140® prior to acetaminophenadministration.

Treatment Groups	Urea (mg/dl) Days of Sampling			
	0	7	14	21
Group 1	10.7±0.7	11.0±0.8	10.5±0.4	11.0±0.9
Group 2	12.1±0.6	15.2±1.0^{bd}	8.3±1.4^{a}	9.3±0.3^{ac}
Group 3	11.1±0.8	14.2±1.5^{bd}	9.6±0.9	8.9±1.1
Group 4	12.0±1.1	28.6±1.6^{bd}	12.5±0.8^{b}	12.5±0.4

Group 1 = Control, distilled water administered dog .Group 2 = Silybon 140® 100 mg/kg pre-treated dogs followed by acetaminophen administration. Group 3 = Silybon 140® 200 mg/kg pre-treated dogs followed by acetaminophen administration. Group 4 = acetaminophen only administered group. Significantly decrease along column a; significantly decrease along row c; significantly increase along column b; significantly increase along column d

Table 10. Mean ± SEM Creatinine Concentration of dogs pre-treated with Silybon 140® prior to acetaminophen administration.

Treatment Groups	Creatinine(mg/dl) Days of Sampling			
	0	7	14	21
Group 1	2.2±2.3	2.2±0.3	2.1±0.1	2.1±0.2
Group 2	2.3±0.2	2.1±0.3	1.9±0.1	1.7±0.1
Group 3	2.3±0.2	2.0±0.4	1.4±0.3^{ac}	1.6±0.3
Group 4	2.2±0.3	2.8±0.2^{b}	2.7±0.1^{b}	2.7±0.3

Group 1 = Control, distilled water administered dog. Group 2 = Silybon 140® 100 mg/kg pre-treated dogs followed by acetaminophen administration. Group 3 = Silybon 140® 200 mg/kg pre-treated dogs followed by acetaminophen administration. Group 4 = acetaminophen only administered group. Significantly decrease along column a; significantly decrease along row c; significantly increase along column b; significantly increase along column d

Table 11. Mean±SEM Total Bilirubin Concentration of dogs pre-treated with Silybon 140® prior to acetaminophen administration.

Treatment Groups	Total Bilirubin (mg/dl) Days of Sampling			
	0	7	14	21
Group 1	0.50±0.2	0.48±0.1	0.45±0.2	0.43±0.1
Group 2	0.56±0.1	0.41±0.1	0.30±0.1	0.29±0.1
Group 3	0.43±0.04	0.42±0.3	0.33±0.2	0.31±0.2
Group 4	0.27±0.1	0.76±0.2^{bd}	0.77±0.2	0.84±0.3^{b}

Group 1 = Control, distilled water administered dog. Group 2 = Silybon 140® 100 mg/kg pre-treated dogs followed by acetaminophen administration. Group 3 = Silybon 140® 200 mg/kg pre-treated dogs followed by acetaminophen administration. Group 4 = acetaminophen only administered group. Significantly decrease along column a; significantly decrease along row c; significantly increase along column b; significantly increase along column d

3.3 Effects of Silybon 140®on the Gross and Histopathology of Acetaminophen Treated Dogs

The histopathological changes observed in the liver and the kidney of acetaminophen (500mg/kg) and Silybon 140 plus acetaminophen treated dogs were similar except for the differences in the severity of the lesions. The major lesions observed were diffused hepatocellular coagulative necrosis and marked infiltration of interlobular septum and liver parenchyma by mononuclear cells mainly lymphocytes and macrophages **(Figs. 1- 2).** There were distorted hepatic cords showing different levels of hydropic degeneration. The group treated with Silybon 140® (200mg/kg) prior to acetaminophen administration and the control group showed normal liver architecture **(Figs. 3-4).** The kidney of the dog treated with 500mg/kg of acetaminophen alone and/or

Silybon 140 (100mg/kg) had multiple foci of necrosis and fibrosis of the glomeruli and interstitial nephritis **(Figs. 5-6).** There was also massive infiltration of lymphocytes and destruction of tubules. The control group and the group treated with Silybon 140® (200mg/kg) prior to acetaminophen treatment did not show the presence of any injury to the kidney **(Figs. 7-8).**

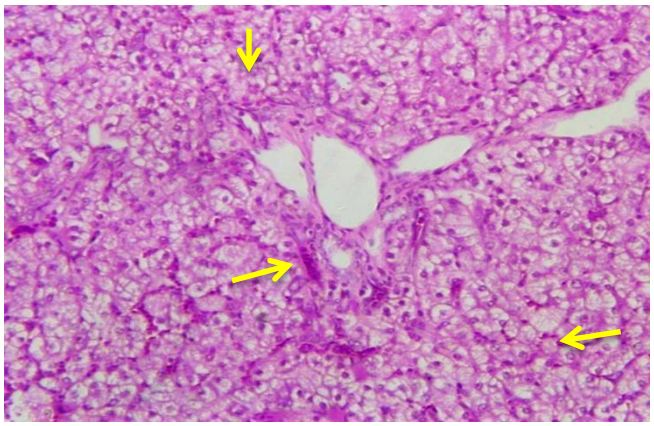

Fig. 1. Liver of dog following administration of acetaminophen (500 mg/kg) showing severe diffused areas of hepatocellular degeneration (arrow) cloudy swelling, hydropic change and distortion of hepatic cords. H&E (x10).

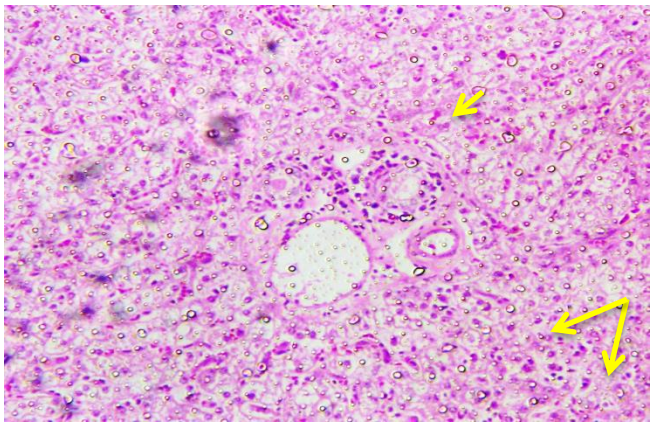

Fig. 2. Liver of dog following acetaminophen (500 mg/kg) plus Silybon 140 (100 mg/kg) showing foci of hepatocellular destruction and infiltration by mononuclear cells made up largely lymphocytes (long arrows) and perivascular infiltration by mononuclear cells made up largely of lymphocytes (short arrows) and diffused hepatocellular degeneration with distortion of hepatic cords. H & E (x10).

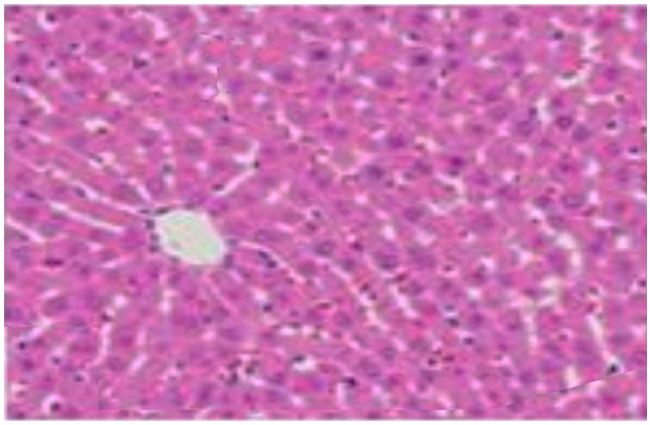

Fig. 3. Photomicrograph of section of the liver of a control dog showing normal liver parenchyma (H&E stain x10).

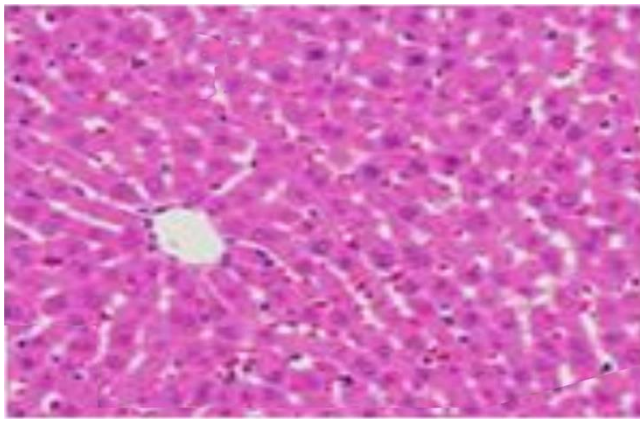

Fig. 4. Liver of dog following administration of acetaminophen (500 mg/kg) plus Silybon 140 (200 mg/kg). Note the normal liver parenchyma (H&E: x10)

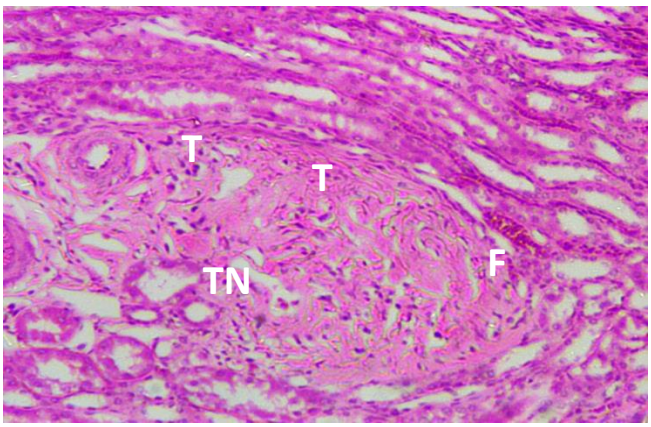

Fig. 5. Kidney of dog treated only with acetaminophen (500 mg/kg) showing areas of tubular fibrosis (T), large focus of massive fibrosis (F) and area of tubular necrosis (TN). H & E (x10)

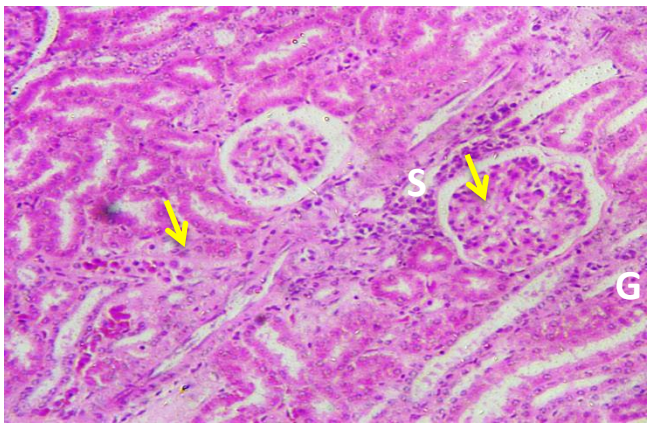

Fig. 6. Kidney of dog treated with 500 mg/kg acetaminophen plus Silybon 140 100 mg/kg showing glomerulonephritis (G) and shrunken glomerulus (S) with interstitial nephritis (arrowed) H & E (x10).

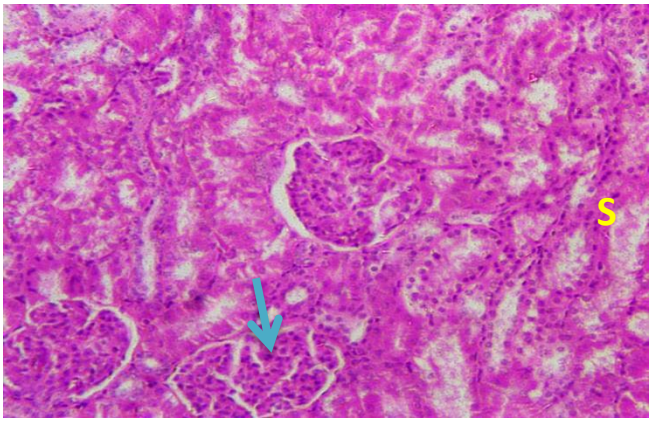

Fig. 7. Photomicrograph of the kidney of a control dog showing normal renal cortex composed of glomeruli, Bowman's capsule, renal tubules and capillary H & E (x10)

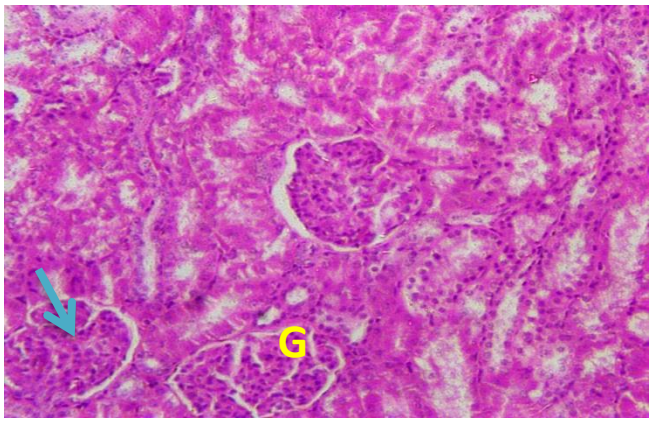

Fig. 8. Kidney of dog treated with acetaminophen (500 mg/kg) plus Silybon 140 (200 mg/kg) showing normal renal cortex. H & E (x10).

4. Discussion

The hematological parameters of dogs treated orally with 500 mg/kg body weight of acetaminophen alone were significantly ($P<0.05$) altered when the pre-treatment and post-treatment values were compared. This observed decrease in hematological parameters (RBC, Hb and PCV) may be an indication of anemia. The anemia could have occurred as a result of hemolysis or inhibition of hematopoiesis in the bone marrow Acetaminophen as observed in the present study significantly ($P<0.05$) affected the values of hematological parameters (RBC, Hb and PCV) of dogs treated with 500 mg/kg alone resulting in anemia. But **Serrano-rodriguez et al. (2019)** reported no changes in the complete blood count dogs treated with acetaminophen at 10 mg/kg and 20 mg/kg. As stated earlier, the anemia may have occurred as a result of inhibition of hematopoiesis or hemolysis of the RBC **(Brown, 1976).** However, treatment with Silybon 140® at the doses of 100 and 200 mg/kg for three days prior to acetaminophen administration appears to have ameliorated the observed anemia due acetaminophen treatment. **Naseer et al. (2016)** observed an improved complete blood count after treatment with Silymarin. Anemia is known to be produced by some drugs and chemicals by inhibiting either the RBC production in the bone marrow or inducing hemolysis of the RBC.

Acetaminophen is known to induce hemolytic anemia in cats **(Allen, 2003).** The administration of acetaminophen to dogs at the dose of 500 mg/kg also resulted in an increased AST level during the three weeks of study. This finding indicates that acetaminophen is injurious to the liver and the skeletal muscles.

ALT is an enzyme found mainly in liver cells. An elevated ALT is regarded as a sensitive index of liver injury **(Tietz, 1994).** In this study, a significant ($P<0.05$) elevation in ALT level occurred from day seven till days twenty-one following acetaminophen administration. Silybon 140® treatment for 5 days prior to acetaminophen administration appears to prevent the hepatic damage induced by acetaminophen. This finding agrees with those of **Pari and Kumar (2002); Nwachujor et al. (1997)** and **Nwachujor et al. (2012),** who have used Silymarin to prevent hepatic damage induced by acetaminophen in rats.

There was also a significant ($P<0.05$) increase in the level of ALP in the dogs treated with acetaminophen alone, when compared to the groups treated with Silybon 140® for 5 days before acetaminophen administration. ALP is an enzyme which mediates some of the complex reactions of bone formation. During bone formation, osteoblasts are known to actively deposit large quantities of ALP in bone matrix. Since the two major sources of ALP are bone and liver, an elevation of ALP immediately direct attention to either a liver problem or bone disease **(Tilkian et al., 1979).** The functional ability of the liver seems to be preserved by the administration of Silybon 140® prior to the administration of acetaminophen to dogs. This was revealed when both doses of Silybon 140® produced reduction in the level of transaminases. Furthermore, treatment with acetaminophen alone in dogs produced increased total bilirubin which was noticeable throughout the study period however the dogs treated with Silybon 140 prior to acetaminophen administration had decreased total bilirubin. Serum bilirubin is one of the most sensitive tests used in the diagnosis of liver diseases. Hyperbilirubinemia may be observed due to increased heme destruction and blockage of biliary tract. Because of the blockage, there is mass inhibition of conjugation reaction, with release of unconjugated bilirubin from damaged and dead hepatocytes **(Wolf et al., 1997).**

The presence of unconjugated bilirubin was not assessed in this study. The serum creatinine and urea levels were significantly ($P<0.05$) increased in the dogs treated with acetaminophen (500mg/kg) alone, especially on day 7 of the study (i.e. 3 days post acetaminophen administration). Similar observations occurred in animals treated with Silybon 140® five days prior to acetaminophen administration. The increases decreased by 14 and 21 days of study. The lowering of serum creatinine and urea levels may be due to the clearance of creatinine and urea from the blood. Serum creatinine determination is one of the means of testing for renal function **(Tilkian et al., 1979).** This significant

improvement in the hematology and serum biochemical parameters could be studied in a clinical trial of Silybon 140 for several other forms hepatic injuries.

5. Conclusion

Acetaminophen administered orally caused anemia, hepatotoxicity and renal toxicity in the Nigerian local dogs but treatment with Silybon 140 improved the anemia since it has flavonoids that provides hematinic activities and effectively reversed the hepatic and renal toxicities produced by acetaminophen. Thus Silybon 140 is effective in management of hepatic and renal toxicities in Nigerian local dogs.

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7. Conflict of interest

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

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