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Original article

Ameliorative effects of piroxicam on perchloric acid-induced thyroid gland hormones disruption in male rats

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ABSTRACT

Background: The incidence of goitre has increased world-wide, with African countries responsible for over 28 % of the cases. The danger of perchloric acid among males working in the factories has been on the increase. Hence mitigating effects of piroxicam on perchloric acid-induced thyroid hormones disruption was studied in male Wister albino rats.

Methods: A total of 19 rats (17 males; 2 females) that weighed 145.0 ± 20.5 g were used for the study. Four rats were used for determination of median lethal dose (LD_{50}) of perchloric acid, whereas 15 rats divided into three groups of five each, were used for induction of thyroid hormones disruption, using 100 mg/kg of perchloric acid for a period of two weeks. Thereafter group II and III rats were treated with 2.5 and 5.0 mg/kg of piroxicam, respectively. Three millilitres of blood were collected from all the rats for determination of thyroid hormones, haematological and biochemical parameters. Brain, liver, spleen, lung, kidney and heart were harvested for allometry and histopathology.

Results: Findings have shown that LD_{50} of perchloric acid was 4207.5 ± 457.6 mg/kg body weight. Piroxicam significantly decreased ($p < 0.05$) thyroid stimulating hormone, but increased free triiodothyronine and thyroxine respectively. Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and monocytes were increased significantly ($p < 0.05$). Urea, creatinine, haematocrit, erythrocytes, leucocytes, lymphocytes, neutrophils, platelets and eosinophils counts were decreased significantly ($p < 0.05$). Allometric parameters were significantly increased ($p < 0.05$). Histopathology revealed perivascular cuffs of brain, zenker's necrosis with mononuclear cells infiltration of myocardium, congestion of central vein of liver, mononuclear cells infiltration of the kidney, lymphoid tissue aggregation of white pulp, congestion and thickness of the lungs were observed in the rats administered piroxicam.

Conclusion: Hence piroxicam ameliorates thyroid hormones disruption, induces liver enzymes, modulates humoral immune system, enhances renal function and decreases allometric parameters of brain, lung and liver, as evidenced by histopathological lessons.

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1. Introduction

Male rodents are more sensitive to piroxicam and hydrocortisone as compared to female rodents (Saganuwan and Orinya, 2016). This may be due to higher ability for activation of metabolic process (NIEHS, 2000). The interest on toxicological effect of potent toxicant on living organism has increased (Forni, 2014). Hence laboratory

dose is the point-of-departure for assessment of toxic chemicals. The increased attrition rate in development of a new anticancer is exasperating. Induction of thyroid disruption by fipronil is via increased elimination of thyroxine (T4) mediated by activation of hepatic enzyme. Fipronil sulfone, the metabolite of fipronil might have contributed to the disruption (Julien et al., 2009). High fat diet caused hypothyroidism mitigated by metformin (El-Sayed and Ibrahim, 2020). More so glucagon-like peptide may increase the risk of thyroid cancer. Perchloric acid could cause benign and malignant thyroid tumours and immunotoxicity. The oral median lethal dose (LD_{50}) of perchloric acid was 1100 and 250 mg/kg in rats and dogs respectively. The subcutaneous LD_{50} in mice was 250 mg/kg. Chronic injection of perchloric acid of 600–1200 mg/kg was used to treat grave's disease, in which immunoglobulin binds to thyroid stimulating hormones receptor,

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resulting in hypothyroidism (Baran and Gad, 2014). Hence the present study aims to assess mitigating effects of piroxicam on perchloric acid-induced thyroid gland hormones disruption in male rats.

2. Materials and methods

2.1. Animals

A total of 2 female and 17 male rats weighed 145.0 ± 20.5 g were used for the study. The rats were obtained from a breeder in Makurdi Metropolis, Nigeria and housed in the Department of Veterinary Physiology and Biochemistry laboratory, Federal University of Agriculture, Makurdi, Benue State, Nigeria. Feed Growers Marsh® and water were provided *ad libitum*. The animals were handled according to the international guiding principle on the use of animals (NIH, 1985), and the recommendation of the Department of Veterinary Pharmacology and Toxicology, Federal University of Agriculture Makurdi Ethical Committee on the use of animals given the permit number (2021003).

2.2. Drugs

Perchloric acid manufactured in Gujarat India, with batch number (090920) and piroxicam capsule were used for the study.

2.3. Acute toxicity study

Randomized control trial was adopted for the study. Acute toxicity study of perchloric acid was carried out on rats, divided into two groups of two males and females. The first male and female each, was administered oral 5000 mg/kg body weight of perchloric acid and observed for 48 h. After death of the two rats, the 2nd male and female rats were administered 3415 mg/kg body weight of perchloric acid, and observed for 48 h and thereafter for 12 days for observation of toxicity signs (Saganuwan and Orinya, 2016).

2.4. Induction of thyroid gland-hormones disruption

Fifteen male rats were divided into three groups of five each. All the groups were treated orally, with 100 mg/kg of perchloric acid for a period of 14 days, for induction of thyroid gland hormones disruption. Pretreatment blood samples were collected before the induction.

Table 1
Effects of piroxicam on perchloric acid-induced thyroid hormones disruption in rats.

Dose of Piroxicam (mg/kg)	Thyroid stimulating hormones (piu/ml)		
	Period of treatment (days)		
	0	14	28
0.0	1.00 ± 0.00	1.04 ± 0.02 ^a	0.84 ± 0.22
2.5	1.02 ± 0.01	0.51 ± 0.00 ^{bd}	0.49 ± 0.01 ^{bd}
5.0	1.00 ± 0.00	0.51 ± 0.01 ^{bd}	0.52 ± 0.27 ^{bd}
	Free triiodothyronine (pg/ml)		
0.0	2.67 ± 0.00	1.37 ± 0.07 ^b	1.86 ± 0.14 ^b
2.5	2.20 ± 0.32	1.37 ± 0.02 ^b	2.25 ± 0.31 ^a
5.0	2.85 ± 0.17	1.50 ± 0.19 ^b	2.90 ± 0.17 ^{ac}
	Thyroxine (mg/ml)		
0.0	0.78 ± 0.07	2.58 ± 0.25 ^a	4.65 ± 0.38 ^a
2.5	0.77 ± 0.07	2.71 ± 0.24 ^a	4.34 ± 0.21 ^{ad}
5.0	0.74 ± 0.04	3.12 ± 0.51 ^{ac}	5.70 ± 0.45 ^{ac}

Keywords: a = significantly higher along the row; b = significantly lower along the row; c = significantly higher along the column; d = significantly lower along the column: level of significance ($p < 0.05$).

2.5. Mitigation of thyroid gland hormones disruption

After the collection of blood samples on day 14, piroxicam was administered at dose rate of 2.5 and 5 mg/kg body weight to group 11 and 111 rats respectively, for a period of 14 days. The experiment was terminated on day 29 of the experimentation.

2.6. Collection of blood samples

One milliliter (1 ml) and 2 ml each of blood was collected into ethylene diamine tetracetic acid bottles and plain bottles for haematology and serum biochemistry respectively. Conversion of pmol to mg/dl was by multiplication of 0.0113, whereas that of mg/dl to pmol was by multiplication of 88.4 respectively.

2.7. Determination of haematological parameters

The red blood cells count (RBCs), packed cell volume, total leucocytes count, differential leucocytes count and platelets count were performed according to the established methods (Cheesbrough, 1998).

2.8. Determination of biochemical parameters

Serum creatinine was estimated by the Modified Jaffe's method (Blass et al., 1974). Plasma creatinine was calculated using the formula given as follow: $Scr = Pcr / 1440 \times 1000$ ml (Saganuwan,

Table 2
Effects of piroxicam on liver enzymes and renal parameters of perchloric acid-induced thyroid hormones disruption in rats.

Dose of Piroxicam (mg/kg)	Alanine aminotransterase (iu/l)		
	Period of treatment (days)		
	0	14	28
0.0	92.30 ± 7.0	131.87 ± 16.35 ^a	122.33 ± 7.93 ^a
2.5	70.90 ± 46.00 ^d	47.95 ± 26.05 ^{bd}	119.13 ± 11.47 ^a
5.0	140.70 ± 27.30 ^c	144.60 ± 35.80	124.10 ± 20.90
	Asparatate aminotransterase (iu/l)		
0.0	172.4 ± 62.1	283.60 ± 32.32 ^a	215.55 ± 25.49 ^a
2.5	132.5 ± 41.0 ^d	216.30 ± 3.90 ^{ad}	190.33 ± 37.30 ^a
5.0	327.3 ± 94.7 ^c	182.23 ± 60.40 ^{bd}	227.20 ± 110.40 ^b
	Alkaline phosphatase (iu/l)		
0.0	8.9 ± 3.2	8.78 ± 2.38	24.54 ± 5.30 ^a
2.5	11.7 ± 2.0	14.10 ± 3.91 ^c	42.07 ± 7.37 ^{ac}
5.0	6.8 ± 2.1	9.58 ± 4.17 ^a	22.80 ± 0.10 ^a
	Renal parameters		
Dose of Piroxicam (mg/kg)	Urea (mmol/l)		
	Period of treatment (days)		
	0	14	28
0.0	13.4 ± 0.7	6.20 ± 0.19 ^b	10.66 ± 1.74 ^b
2.5	13.6 ± 1.2	6.44 ± 0.21 ^b	7.25 ± 0.95 ^b
5.0	14.9 ± 2.0	6.23 ± 0.16 ^b	4.34 ± 1.33 ^b
	Serum creatinine (umol/L)		
0.0	100.0 ± 13.4	95.08 ± 4.57	71.30 ± 10.34 ^b
2.5	105.5 ± 10.1	114.87 ± 7.65	57.67 ± 7.57 ^b
5.0	92.8 ± 5.7	98.40 ± 10.78	69.00 ± 5.25 ^b
	Plasma creatinine (umol/L)		
0.0	144.0 ± 19.3	136.92 ± 6.58	102.67 ± 14.89 ^b
2.5	157.9 ± 14.5	165.41 ± 11.02	83.04 ± 10.90 ^{bd}
5.0	133.6 ± 8.2	141.70 ± 15.52	99.36 ± 7.56 ^b

Keywords: a = significantly higher along the row; b = significantly lower along the row; c = significantly higher along the column; d = significantly lower along the column: level of significance ($p < 0.05$).

Table 3
Effects of piroxicam on hematological parameters of perchloric acid-induced thyroid hormones disruption in rats.

Dose of Piroxicam (mg/kg)	Hematocrit (%)		
	Hematocrit (%)		
	Period of treatment (days)		
	0	14	28
0.0	44.20 ± 3.37	38.80 ± 0.73 ^b	40.75 ± 0.85 ^{bd}
2.5	46.80 ± 1.53	42.25 ± 1.03 ^{bc}	36.00 ± 1.00 ^{bd}
5.0	41.80 ± 4.09	38.25 ± 0.75 ^b	43.50 ± 0.41 ^{bc}
	Ermthrocytes counts (x10 ¹² /L)		
0.0	4.91 ± 0.37	4.31 ± 0.08	4.52 ± 0.09
2.5	5.20 ± 0.17	4.69 ± 0.12 ^b	4.00 ± 0.11 ^b
5.0	5.00 ± 0.45	4.25 ± 0.08 ^b	4.83 ± 0.04 ^b
	Leucocytes counts (x10 ⁹ /L)		
0.0	8.34 ± 1.18	7.96 ± 1.00	4.30 ± 1.16 ^b
2.5	5.02 ± 0.72	4.37 ± 1.30	3.57 ± 0.55 ^b
5.0	4.64 ± 1.29	5.88 ± 1.64	7.55 ± 2.05
	Monocytes (%)		
0.0	7.60 ± 0.51	7.60 ± 1.21	7.50 ± 0.96
2.5	6.60 ± 0.68	10.25 ± 0.25 ^{ac}	9.33 ± 1.33 ^a
5.0	5.00 ± 1.67	7.25 ± 0.76	9.50 ± 0.50 ^a
	Lymphocytes (%)		
0.0	47.20 ± 4.62	57.20 ± 5.75	70.25 ± 2.06 ^a
2.5	55.20 ± 2.96	57.00 ± 3.49	48.00 ± 13.08 ^{bd}
5.0	56.60 ± 5.49	48.75 ± 3.59 ^b	61.00 ± 2.00 ^d
	Neutrophils (%)		
0.0	44.40 ± 35.24	40.60 ± 5.35 ^b	22.25 ± 1.93 ^b
2.5	36.60 ± 2.46	32.75 ± 3.52 ^a	42.67 ± 12.03 ^c
5.0	36.40 ± 3.84	75.00 ± 3.28 ^{ac}	29.00 ± 2.00 ^c
	Platelets (x10 ¹² /L)		
0.0	398.00 ± 35.24	159.00 ± 16.31 ^b	337.25 ± 82.12
2.5	605.80 ± 103.67	177.50 ± 16.14 ^b	341.67 ± 27.29 ^b
5.0	432.20 ± 54.74	135.00 ± 9.79 ^b	350.00 ± 195.00 ^b
	Eosinophils (%)		
0.0	1.00 ± 0.55	0.60 ± 0.24 ^b	0.00 ± 0.00 ^b
2.5	1.20 ± 0.20	0.00 ± 0.00 ^{bd}	0.00 ± 0.00 ^b
5.0	2.00 ± 0.71	0.25 ± 0.25 ^{bd}	0.50 ± 0.00 ^b
	Basophils (%)		
0.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
2.5	0.40 ± 0.24	0.00 ± 0.00	0.00 ± 0.00 ^c
5.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Keywords: a = significantly higher along the row; b = significantly lower along the row c = significantly higher along the column d = significantly lower along the column: level of significance (p < 0.05).

Table 4
Allometric scaling of brains, heart, liver, kidney, lung and spleen of rats treated with perchloric acid and piroxicam.

Parameters	Treatment	Groups (mg/kg)	
	0	2.5	5.0
Body weight (g)	150.5 ± 30.9	112.0 ± 5.06	143.5 ± 11.5
Brain (g)	1.6 ± 0.0	1.6 ± 0.1	1.4 ± 0.05
Brain length (cm)	1.9 ± 0.0	2.2 ± 0.1 ^a	1.7 ± 0.1 ^b
Brain width (cm)	1.5 ± 0.1	1.3 ± 0.1 ^b	1.3 ± 0.1 ^b
Heart (g)	0.41 ± 0.07	0.42 ± 0.06	0.63 ± 0.02 ^a
Length (cm)	1.28 ± 0.14	1.30 ± 0.00	1.60 ± 0.20 ^a
Width (cm)	0.65 ± 0.06	0.70 ± 0.10	1.00 ± 0.00 ^a
Liver right lobe (g)	2.48 ± 0.38	2.68 ± 1.0 0 ^a	2.47 ± 0.47
Left lobe (g)	1.63 ± 0.52	1.76 ± 0.60 ^a	2.50 ± 0.08
Left lobe length (cm)	2.87 ± 0.03	2.65 ± 0.45 ^b	3.25 ± 0.35
Right lobe length (cm)	3.07 ± 0.23	2.70 ± 0.00 ^b	3.00 ± 0.00 ^a
Width of right lobe (cm)	1.70 ± 0.15	1.85 ± 0.45 ^a	1.40 ± 0.10 ^b
Width of Left lobe (cm)	1.50 ± 0.06	1.55 ± 0.60	1.85 ± 0.15 ^b
Left Kidney (g)	0.49 ± 0.04	0.45 ± 0.05	0.52 ± 0.02
Right kidney (g)	0.46 ± 0.06	0.48 ± 0.06	0.53 ± 0.02
Length of right kidney(cm)	1.60 ± 0.06	1.40 ± 0.15	1.75 ± 0.05 ^a
Length of left kidney (cm)	1.57 ± 0.07	1.47 ± 0.24	1.75 ± 0.25 ^a
Width of right kidney (cm)	0.60 ± 0.06	0.77 ± 0.22	0.65 ± 0.05
Width of left kidney (cm)	0.50 ± 0.06	0.70 ± 0.10 ^a	0.65 ± 0.15 ^a
Spleen (g)	0.40 ± 0.06	0.39 ± 0.10	0.73 ± 0.08 ^a
Length of spleen (cm)	3.15 ± 0.23	2.63 ± 0.09 ^b	3.55 ± 0.35 ^a
Width of spleen (cm)	0.40 ± 0.04	0.90 ± 0.35	0.65 ± 0.25 ^a

(continued on next page)

Table 4 (continued)

Parameters	Treatment		
	0	2.5	5.0
Right lung (g)	0.55 ± 0.09	0.67 ± 0.18	0.68 ± 0.03
Allometric ratio	0.004 ± 0.003	0.006 ± 0.036 ^a	0.005 ± 0.003 ^a
Left lung (g)	0.53 ± 0.05	0.51 ± 0.12	0.51 ± 0.01
Allometric ratio	0.004 ± 0.002	0.005 ± 0.024 ^a	0.004 ± 0.001
Length of right lung (cm)	2.22 ± 0.36	2.07 ± 0.23	2.45 ± 0.05 ^a
Width of right lung (cm)	1.03 ± 0.08	1.00 ± 0.021 ^d	1.05 ± 0.05
Length of left lung (cm)	2.18 ± 0.27	1.83 ± 0.23	2.05 ± 0.05 ^b
Width of left lung (cm)	1.00 ± 0.10	1.10 ± 0.12 ^d	1.35 ± 0.05 ^{ad}

Keywords: a = significantly higher along the row; b = significantly lower along the row; c = significantly higher along the column; d = significantly lower along the column; level of significance (p < 0.05).

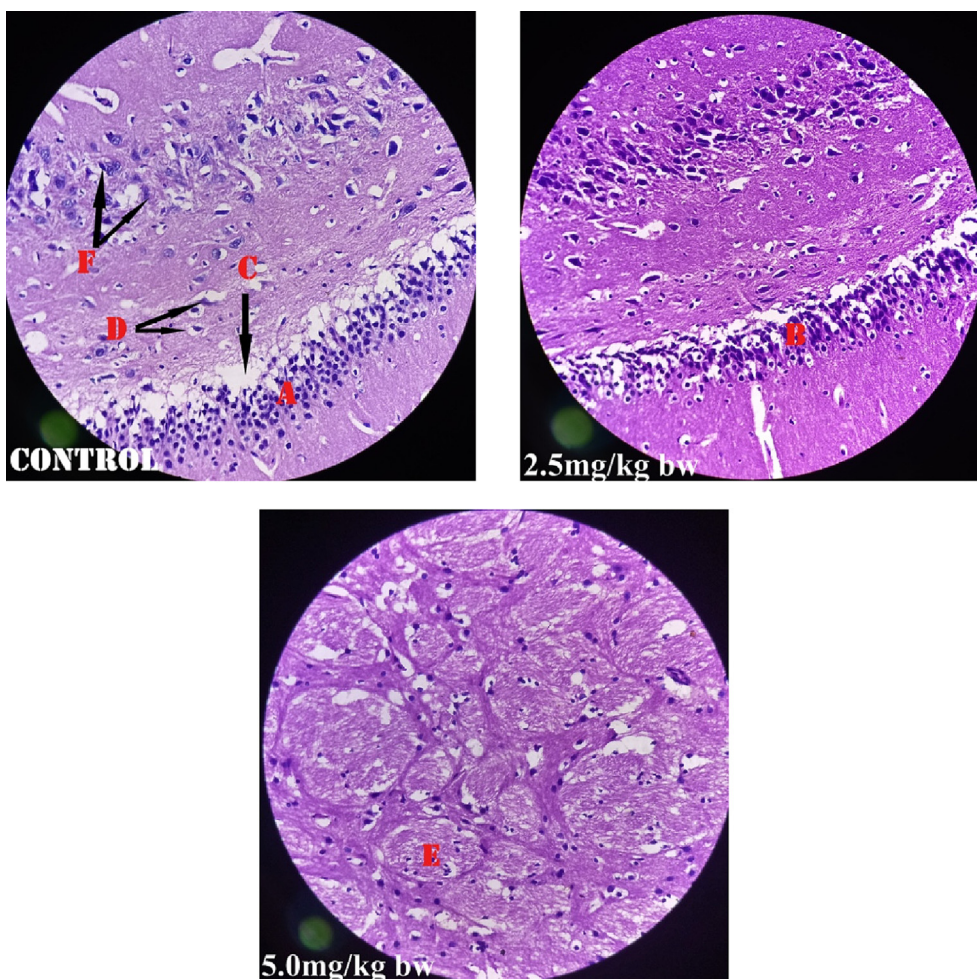


Fig. 1. Shows perivascular cuffs (A&B), under perivascular space (C), glial cells (D), peripheral nerve (E) and mononuclear cells (F).

2018). Serum urea (Talke and Schubert, 1965), serum aspartate aminotransferase (Bergmeyer et al., 1976), and serum alanine aminotransferase (Henley, 1980) and alkaline phosphatase (Roy, 1970) were determined according to the established methods.

2.9. Determination of thyroid hormones

The quantitative determination of total thyroxine, free triiodothyronine and thyroid stimulating hormone concentration in

serum was done by a Microplate Enzyme Immunoassay method (Chopra et al., 1971; Hopton and Harrap, 1986).

2.10. Histopathology

The survived animals were sacrificed using 100 mg/kg of intraperitoneal phenobarbitone. The brain, lungs, kidneys, spleen, heart, and liver were collected for allometry and thereafter fixed with 10% formalin for histopathology. The sampled organs

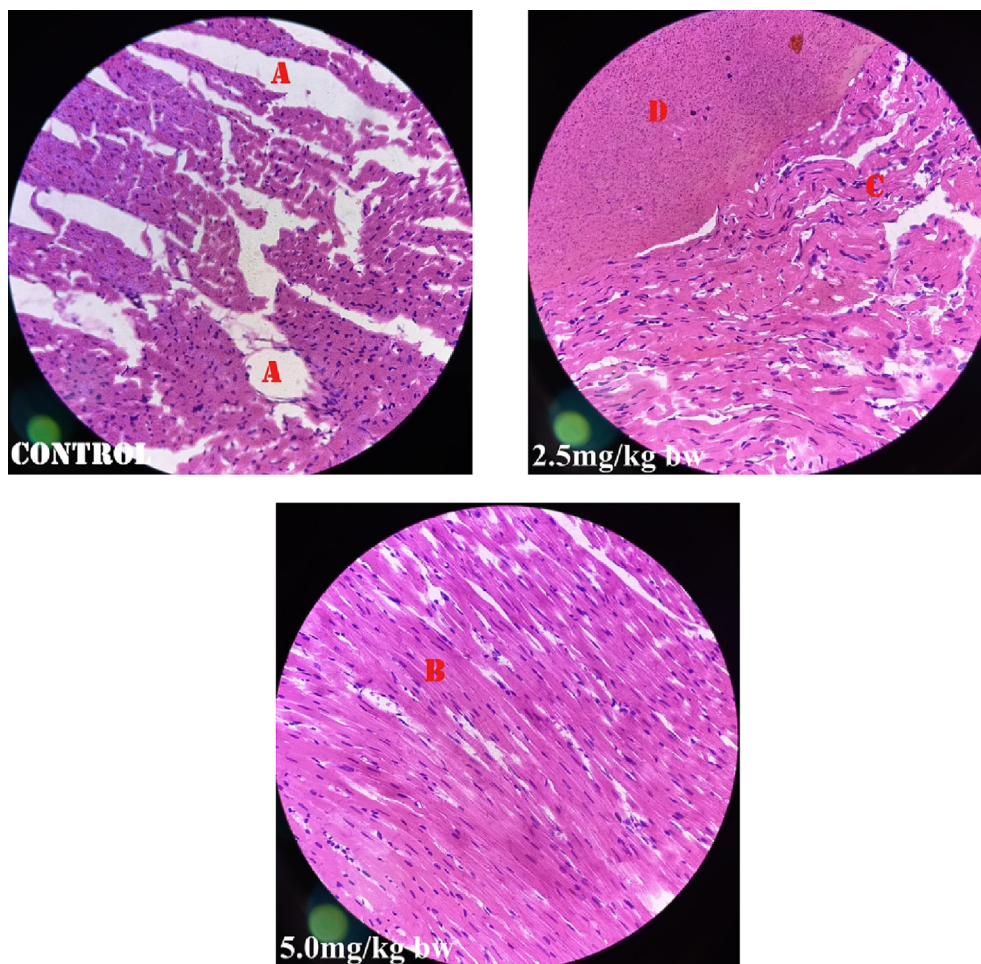


Fig. 2. Shows Zenker's necrosis (A), normal myocardium (B&D), and Zenker's necrosis with mild mononuclear cellular infiltration (C).

obtained were dehydrated using 70, 95 and 100% ethanol. The embedded tissues were stained with hematoxyline and Eosin (H&E), observed under microscope (x100) and photographed using Vanox Olyonpus Microscope (Drury et al., 1976).

2.11. Statistical analyses

All data generated have been presented in mean \pm standard error of mean. The data were analyzed using two-way analysis of variance and least significant difference was detected at 5% level (Daniel, 2010).

3. Results

Acute toxicity study of perchloric acid revealed median lethal dose (LD50) of 4207.5 ± 457.6 mg/kg body weight. The male and female rat administered 5000 mg/kg died within 14 days, after the administration of the chemical. Meanwhile, male and female rat administered 3415 mg/kg body weight survived the dose after 14 days. The effects of piroxicam on perchloric acid-induced thyroid hormones disruption in male rats is presented in the Table 1. Groups treated with piroxicam showed significant decrease ($p < 0.05$) in thyroid stimulating hormones (TSH) and free triiodothyronine (fT3) whereas thyroxine (T4) increased significantly ($p < 0.05$), respectively (Table 1). Swelling of thoracic outlet and

muscle of hindlimb were observed in some rats of group 2 and 3. Alanine amino transferase (AAT), aspartate amino transferase (AST) and alkaline phosphatase were significantly increased ($p < 0.05$) in the group administered perchloric acid only. The levels of urea, serum creatinine and plasma creatinine were significantly ($p < 0.05$) decreased (Table 2).

Haematocrit, erythrocytes and leucocytes counts were significantly ($p < 0.05$) decreased in perchloric acid and piroxicam treated groups, whereas monocytes counts were significantly increased ($p < 0.05$) in the groups treated with piroxicam respectively. Lymphocytes and neutrophils were significantly ($p < 0.05$) increased and decreased in the perchloric acid treated group respectively. Platelets and eosinophils were significantly decreased in all the groups ($p < 0.05$) except basophils, which decreased in 2.5 mg/kg piroxicam treated group (Table 3). The weight, width and length of heart were significantly ($p < 0.05$) increased. The significant increase in the weight and decrease ($p < 0.05$) in the length of right and left lobes of the liver were observed in the group administered piroxicam. However, the width of the right and left lobes of the liver was significantly ($p < 0.05$) decreased in the group administered piroxicam. Nevertheless the length and width of right and left kidneys, weight, length and width of spleen as well as length and width of right lung were significantly increased ($p < 0.05$) in the group administered piroxicam, whereas length of the lung was significantly decreased ($p < 0.05$). Meanwhile

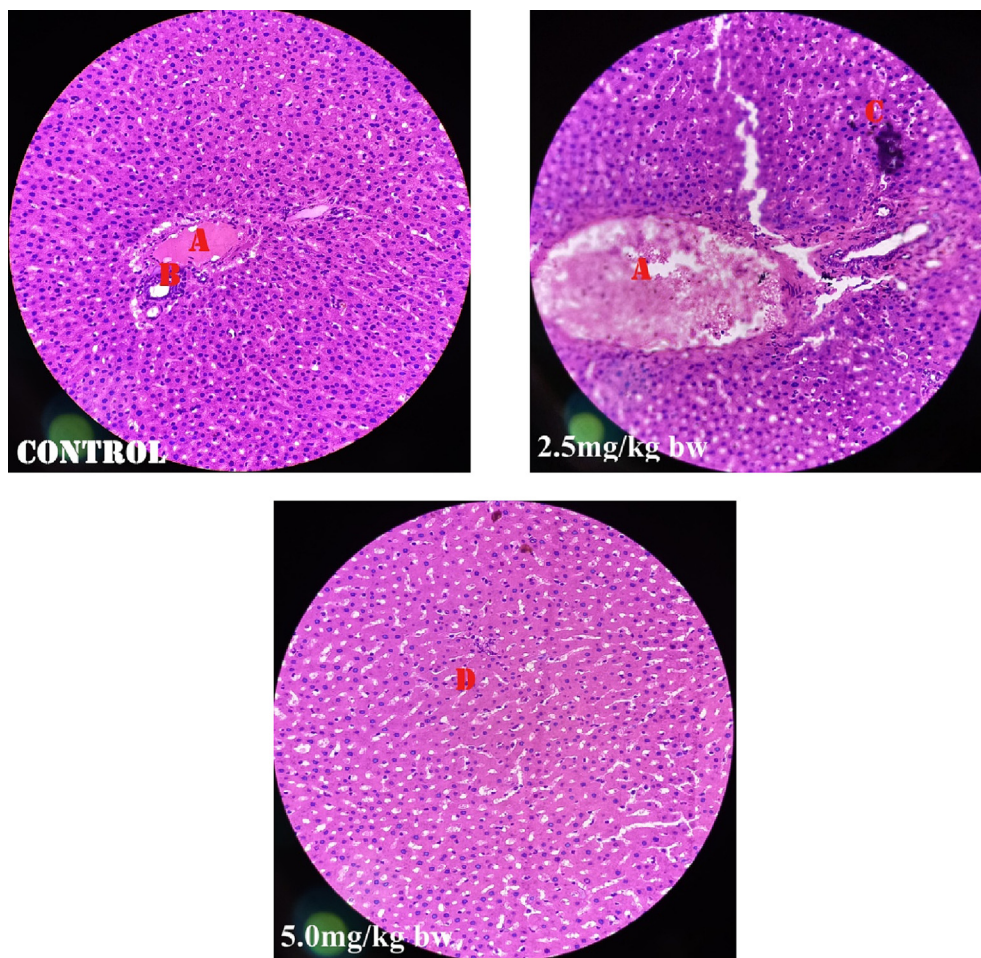


Fig. 3. Shows congestion of central vein (A), normal portal tract (B), mononuclear cell infiltration (C), whereas (D) is normal hepatocytes.

allometric ratio of the brain was significantly increased ($p < 0.05$) in the group administered piroxicam. Allometric ratios of the liver, heart, kidney, lung and spleen were increased significantly ($p < 0.05$) in the group administered 5 mg/kg of piroxicam (Table 4).

3.1. Histopathology

Fig. 1 shows perivascular cuffs and normal neurological cells of the male rats administered perchloric acid and perchloric acid/piroxicam respectively. Fig. 2 shows zenker's necrosis of myocardial cells of the rats administered, perchloric acid only. Congestion of the central vein and mononuclear cells infiltrations were observed in the group administered, perchloric acid and piroxicam (Fig. 3). Normal medullary rays, normal glomeruli surrounded by Bowman's capsule and distal convoluted tubules with mononuclear cells infiltration were observed in the group administered perchloric acid and piroxicam, respectively (Fig. 4). White pulp with aggregation of lymphoid tissue and red pulp with aggregation of both white and red pulps, were observed in all the three groups (Fig. 5). Wide alveolar space was observed in the group administered piroxicam. Congestion and thickness of alveolar sac were observed in the group administered perchloric acid, whereas thick narrow air sacs with congested artery and large alveolar sac with thick wall comprising pneumocytes were observed in the group administered piroxicam, respectively (Fig. 6).

Haemorrhages in the lung, spleen, kidney and heart, paleness of small and large intestine, shrinking of the stomach and diarrhoea

where observed in the group administered 5 mg/kg body weight of piroxicam plus perchloric acid 100 mg/kg. Haemorrhages were observed in the brain of the group administered 2.5 mg/kg of piroxicam plus perchloric acid. The animals were emaciated and the average weight reduced from 163.3 ± 10.5 g to 135.5 ± 11.8 g translating to 17 % of total body weight loss.

4. Discussion

The calculated LD₅₀ of 4207.5 ± 457.6 mg/kg body weight of perchloric acid shows that the chemical is not safe. The finding agrees with the report of Saganuwan and Orinya (2016) indicating that toxic agent with LD₅₀ < 5000 mg/kg is not safe. Hypnosis, calmness and dullness observed in the present study show that perchloric acid may depress central nervous system. Meanwhile, the increased T₄ observed in all the groups on day 14 and 28 showed that both perchloric acid and piroxicam could cause increased thyroxine secretion. Perchlorate blocks the transport of iodide from entry into thyroid gland leading to iodine deficiency, which stimulates production of thyroid stimulating hormones (TSH) by the pituitary gland, invariably causing goitre. Perchlorate (HClO₄) absorbed through gastrointestinal tract was excreted in the urine within 48 h, with elimination half-life of 6.99 h. Goitrogens cause physiologic perturbations of TSH, which can lead to tumor associated with hormonal imbalance (Capen, 1997).

Decrease in triiodothyronine (T₃) and thyroxine (T₄) associated with increased TSH are the biomarkers of exposure to perchlorate.

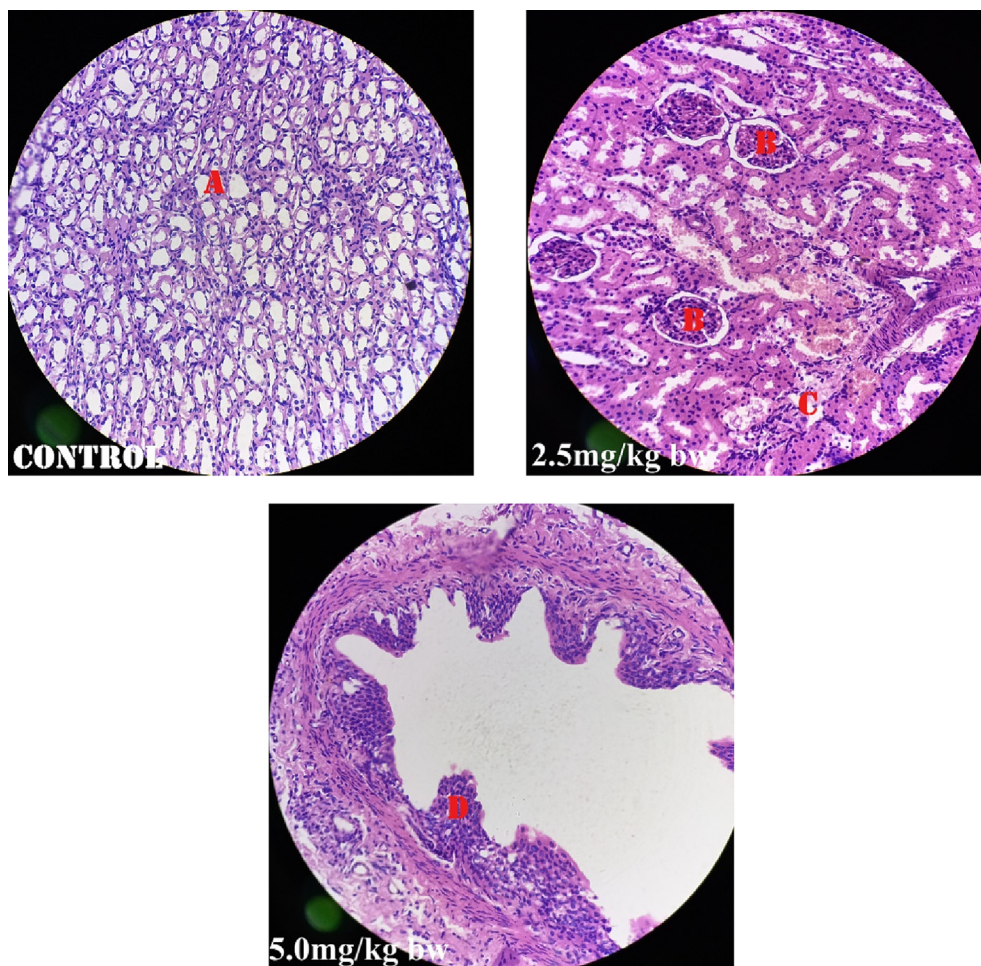


Fig. 4. Shows normal medullary rays (A), glomeruli (B), parenchymal tubule (C), and distal convoluted tubule with mononuclear cells infiltration (D).

Perchlorate is highly persisted in surface water and ground water. Perchloric acid can react with aldehydes, alcohols and ketones, suggesting possible reaction with piroxicam (Baran and Gad, 2005). Iodine deficiency is a risk factor for thyroid cancer and anaplastic thyroid cancer characterized by chronic TSH stimulation and microcarcinoma with lower iodine intake (Zimmermann and Galetti, 2015).

History of thyroid cancer in a family, diet and chemotherapy are predictors of thyroid cancer. Hence theoretic model of antineoplastic could be challenging in cancer chemotherapy (Saganuwan, 2019). Chemically induced cancer in lab animals portends potential hazards in human. TSH-producing pituitary cells undergo hyperplasia and neoplasia (Hill et al., 1998). Iodine ^{131}I administered to patients suffering from thyroid toxicosis may cause failure of thyroid gland. Hepatocellular enzymes induction could cause hypertrophy of thyrotrophs in rats. Polychromated biphenyl (PCB) and prenenoline-160 α carbonitrile (PCN) reduce thyroid hormone levels in rats. The mechanism of action of PCB is via induction of microsomal enzymes. Hyperplasia of parathyroid gland could be caused by X-irradiation on rats (Lindsay et al., 1961). However ^{131}I caused transitional changes to thyroid gland with associated iron T_4 (Rudquist et al., 2017). Thyrotoxicosis is associated with follicular cell hypertrophy, follicular cell hyperplasia, follicular cell adenoma, follicular cell carcinoma, C-cell hyperplasia, C-cell adenoma, C-cell carcinoma, hyperplasia, adenoma and carcinoma (Botts et al., 1991). Thyroid carcinogenesis is via

inhibition of thyroid peroxide and 5-minodeodinate (Hurley et al., 1998). Pyrethrins and sodium phenobarbital caused thyroid hypofunction via induction of cytochrome, CYP2B enzyme in rat liver (Frinch et al., 2006). Perfluorohexane sulfates decreased thyroid hormones level and brain development is sensitive to T_4 (Ramhoj et al., 2020). Progression of thyroid tumor to anaplastic thyroid carcinoma is via p53 in the mouse model of papillary thyroid cancer (Mc Fadden et al., 2014). Thyroid hormones are essential for growth, development of muscular, skeletal and neurological systems, erythropoiesis and thermogenesis. About 99% of thyroid hormones are stored in protein bond-active form. Free T_4 and T_3 are basically active and stimulate feedback mechanism on the hypothalamus and pituitary gland (Thorson, 2014). Disruption of circadian rhythms leads to obesity, diabetes and cardiovascular disease (Bae and Androulakis, 2018). The decrease in renal parameters observed in the present study agrees with the report of Saganuwan (2018), indicating that creatinine could be used for identification of renotoxic agent.

The higher therapeutic effects produced by 2.5 mg/kg of piroxicam as compared to 5 mg/kg shows that piroxicam has hormetic dose response. Saganuwan and Orinya (2016) had earlier reported hormetic dose-response of piroxicam, which is toxico-neurological in rats. The stimulating effect was attributed to the parent drug, piroxicam (Saganuwan, 2017a), whereas depressant effect was attributed to piroxicam metabolites (Saganuwan, 2017a; Saganuwan, 2017b). The hormetic dose response can be due to

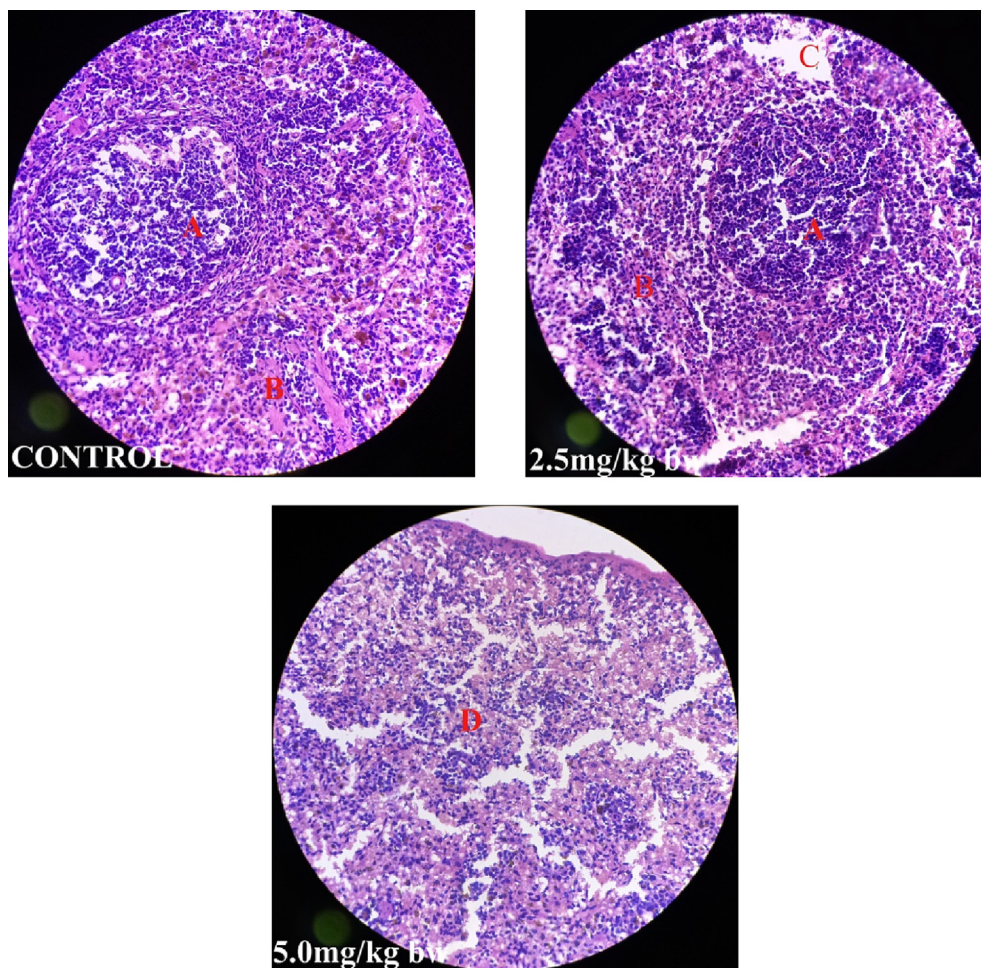


Fig. 5. Shows white pulp with aggregation of lymphoid tissue (A), red pulp with red blood cells and white blood cells (B), degeneration of red pulp (C) and wide spread combined degeneration of white pulp and red pulp (D).

tautomeric behaviour of piroxicam, that makes the drug isomers to be in equilibrium, and changed from acidic to basic or inert form (Saganuwan, 2016). Hormesis is a reversal between high and low doses of physical stressors, phytochemicals, chemicals or biological molecules, and the overall effects could be beneficial or hazardous (Jodynis-Liebert and Kujawska, 2020). Hormetic dose–response is exhibited by endocrine disruptors. The response could be low-moderate, low–high, low or high, represented by Arndt-Schulz curve and generalized adaptive curve. Therefore, the statistical methods used for hormesis are Cedergreen-Ritz-Strenbig model, Brain-Consens model, biologicistic model and An-Johnson-Lovett model (Nweke et al., 2021). Hormesis is constrained by plasticity, though evolutionary, frequent independent, remote-conditioning, risky, synergistic, resilient and requires assessment. The 2.5 mg/kg which produced better effect against disruption of thyroid hormones by perchloric acid is dose–response-time dependent. This type of response requires dose selection, repeated dosing, different inputs, rates and route of drug administration (Gabrielsson et al., 2019). Unfortunately, 25% of medical errors are due to drugs and 14% are attributed to poor drug calculations. The increased basophills, platelets, and eosinopils showed that piroxicam could be used in the treatment of histamine, serotonin and heparin induced reaction, whereas neutropenia observed is suggestive of inflammatory response and lymphocytosis observed may be due to stimulation of thymus. Lymphocytes are produced from B-lymphocytes (Watkins and Levy, 1988). IgA in the body fluid activates the complement

system, 1gE acts on mast cells and basophills which play roles in inflammation and allergy, 1gD is a membrane receptor and 1gC is found on almost every cell (Percus, 2010).

Perivascular cuffs observed in the group of rats administered 100 mg/kg of perchloric acid and 2.5 mg/kg of piroxicam, show that both agents can cause acute and chronic perivascular cuffs, ameliorated by 5.0 mg/kg of piroxicam. The acute cuffs consist of lymphoid cells, and a few macrophages usually observed in nonde-myelinated white matter and in the margin of plaque. The chronic cuffs consist of macrophages, plasma cells and plasmotoid cells seen in the plaques. Macrophages phagocytize myelins and axon (Tanaka et al., 1975), suggesting that piroxicam is a strong immunocytogenic agent. The observed Zenker's necrosis in group administered 100 mg/kg of perchloric acid suggests that the chemical could cause waxy degeneration of heart striated muscle. The degeneration was ameliorated by 2.5 and 5.0 mg/kg of piroxicam. Zenker's necrosis is caused by infiltration of albuminous material from outside heart cells (Wells, 1908). Since lactic acid may cause Zenker's necrosis, perchloric acid may be the incriminating agent in the present study. Congestion of central veins caused by perchloric acid (100 mg/kg), and in addition to 2.5 mg/kg of piroxicam ameliorated by 5.0 mg/kg of piroxicam, shows that both perchloric acid and piroxicam have negative and positive effects on liver. Normal kidney architecture suggests beneficial effect of the two agents on the kidney. The observed dense white pulp and red pulp in the group administered perchloric acid (100 mg/kg) and in addition to 2.5 mg/kg of piroxicam show that the two agents could stimulate

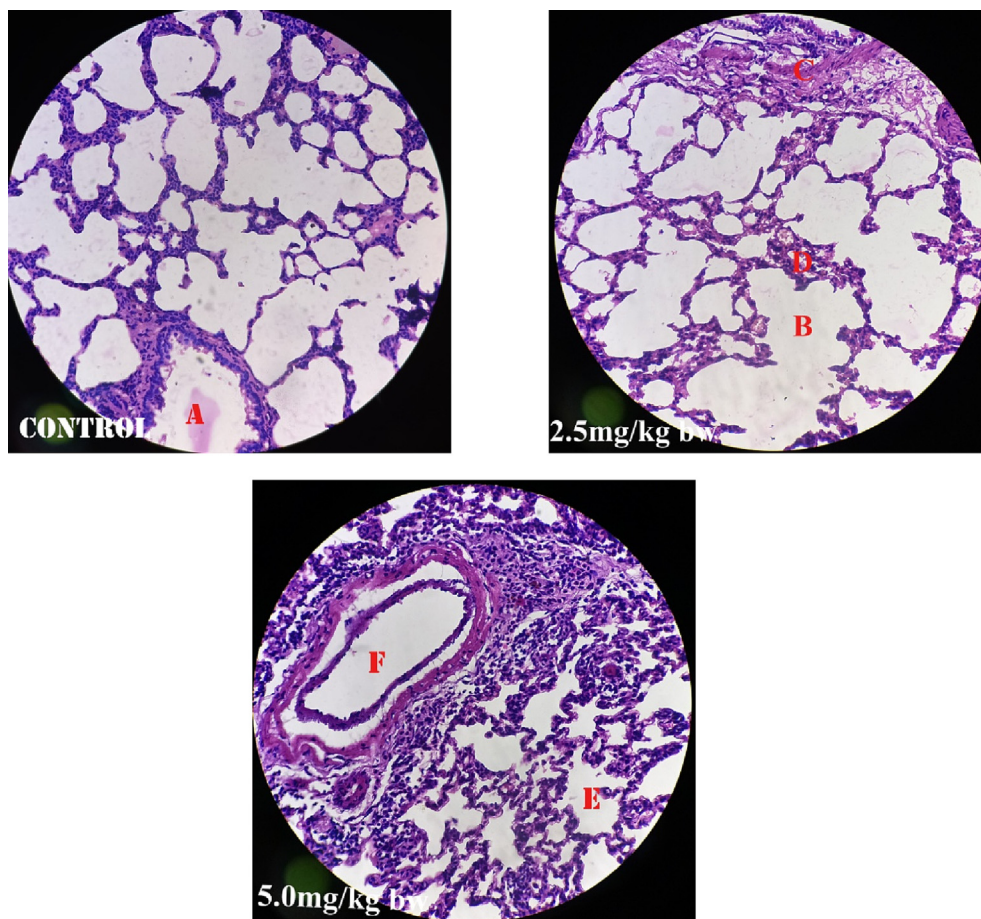


Fig. 6. Shows congested alveolar sac (A), wide alveolar space (B), congested smooth muscle (C), thick pneumocytes (D), thick air sac (E) and congested artery (F).

cellular immune system as evidenced by perivascular cuffing of the leucocytes observed in the brain. The mild degeneration of white and red pulps by piroxicam suggests suppressive potential of lymphoid tissues and erythrocytes.

5. Conclusion

Piroxicam mitigated disruption of thyroid gland hormones at 2.5 and 5 mg/kg body weight. Thyroxine, fT_3 and thyroid stimulating hormone, creatinine, urea, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, mononuclear and polymorphonuclear cells and organ allometry are reliable parameters for identification of thyroid hormones disruptors. Hence piroxicam has neuro-endocrine-immunomodulatory potential and could be used in the management of goitre and treatment of autoimmune diseases.

Declaration of Competing Interest

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author's contributions

SAS designed and carried out the study, did the statistical analysis, wrote and proofread the manuscript.

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Appendix A. Supplementary material

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