Toxico-Neurological Effects of Piroxicam in Monogastric Animals

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ABSTRACT: Piroxicam is a benzothiazine compound with anti-inflammatory, antipyretic, and analgesic properties. Because of the very high efficacy of piroxicam and its increasing use in the treatment of carcinomas in dogs and cats, there is a need for acute toxicity study of piroxicam in monogastric animals and its potential for causing secondary poisoning in puppies. Piroxicam manufactured by Shanxi Federal Pharmaceutical Co, Ltd. was used for this study. Revised up-and-down procedure was used for the estimation of median lethal dose in mouse (259.4 ± 51.9 mg/kg), rat (259.4 ± 69.6 mg/kg), rabbit (707.5 ± 130.8 mg/kg), cat (437.5 ± 128.1 mg/kg), guinea pig (218.7 ± 64.1 mg/kg), monkey (733.3 ± 83.3 mg/kg), broiler (285.3 ± 62.5 mg/kg), hen (638.3 ± 115.4 mg/kg), turkey (707.5 ± 130.8 mg/kg), pigeon (375 ± 55.9 mg/kg), and duck (311.3 ± 46.6 mg/kg). The acute toxicity signs of piroxicam at doses 207.5 mg/kg and above observed in the animals are torticollis, opisthotonos, somnolence, lethargy, diarrhea, gastroenteritis, generalized internal bleeding, anemia, congestion of the lung and liver, flaccid paralysis, cheesy lung, urinary incontinence, engorged urinary bladder, convulsive jerking of the limbs, lying in ventral recumbency, gasping for air, roaring, and death. Three out of six puppies died after being fed the carcasses of poisoned turkey, duck, and hen administered piroxicam at doses of 1000, 415, and 1000 mg/kg, respectively. White flaky cheesy materials observed in turkeys were also observed in the gastrointestinal content of the puppies. Paleness of carcasses, watery crop content, dryness of pericardium, gastroenteritis, intestinal perforation, and whitish pericardium were observed in broilers. There were effusions in thoracic and abdominal cavities as seen in all other carcasses poisoned primarily by piroxicam. Administration of atropine (0.02 mg/kg) led to survival of the remaining puppies. In conclusion, piroxicam is very to moderately toxic in monogastric animals.

KEYWORDS: piroxicam, extrapyramidal effect, median lethal dose, secondary poisoning, puppy

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Introduction

Median lethal dose (LD_{50}) is the amount of a test agent that can cause death in 50% of test animals. The concept of LD_{50} was first introduced in 1927 by Trevan.[1] The up-and-down procedure was adopted in 1981 and revised many times. The method involves the use of less number of animals (4–15), is faster, and its findings are relatively comparable to the findings from other methods.[2]

Piroxicam is a benzothiazine possessing an enolic 4-hydroxy substituent, prostaglandin inhibitor, with active enterohepatic circulation resulting in prolonged half-life (30–85 hours), making it possible for single daily effective dose (20 mg). Elimination is via urine (65%) and faeces (35%). But piroxicam is excreted as glucuronide conjugates of the free acid and hydroxylated metabolites.[3] But the average half-life in human is 45 hours.[4] It is 99% plasma protein bound, and steady-state plasma concentrations are achieved in a week. Rashes, pruritus, edema, and azotemia are also observed in patients taking piroxicam.[5] It is suitable for prolonged use after minor surgery, where 0.5% gel is applied topically. It also interacts with a wide range of drugs and therefore increases the risk of ulcer and bleeding. Such drugs, including oral anticoagulants, corticosteroids, anti-inflammatory drugs, may raise blood levels of lithium and reduce the effects of diuretics and antihypertensive.[6] It is restricted in patients older than 60 years, and 20–40 mg of the drug can be taken once or twice daily.[7] It is a potent drug widely used for chronic inflammatory conditions,[8] with weak cyclooxygenase-2 activity in addition to inhibiting cyclooxygenase I; therefore, adverse effects are reported when large doses are taken over a long period of time.[9] In multiple dosing, the adverse effect profile may be more prominent.[10] This may be due to the enhancement of dissolution rate by electro-spinning technique.[11] When administered orally, it was 100% bioavailable and maximum concentrations (3.1 ± 1.0 hours) were achieved quickly.[12] Piroxicam-β-cyclodextrin has a gastrointestinal safety profile that is better than that shown by uncomplexed piroxicam.[13] It acts as a potent aquaporin-4 inhibitor and renders neuroprotection in focal cerebral ischemia in rats and therefore may be used for the treatment of brain stroke along with other anti-stroke therapeutics.[14]

The reported antitumor activity of piroxicam is not considered a result of direct cytotoxicity, but at high concentration it inhibits the migration of polymorphonuclear

leukocytes, decreases the production of oxygen radicals, and inhibits the function of lymphocytes.[15] The empirical oral dose (0.26 mg/kg) every 24 hours was used to reduce dysuria and pollakiuria in cats with idiopathic lower urinary tract diseases.[16] However, the oral dose of 0.3 mg/kg per day than every 2 days in food relieved pain in dog[17] and showed antitumor activity in dogs with transitional cell carcinoma of the urinary bladder in phase I and phase II clinical trials.[18,19] In 34 dogs treated with piroxicam (0.3 mg/kg per os every 24 hours), tumor responses were seen in two (complete remission) and four dogs (partial remission). Median survival of all the dogs was 181 days; these signs resolved with discontinuation of the drug.[19] Two out of six dogs tolerated piroxicam when given with misoprostol.[20] But when combined with cisplatin in dogs with transitional cell carcinoma, it induced remission more frequently than cisplatin alone, but renal toxicity was more severe and dose limiting.[21] The major metabolic transformation of the drug in human beings is via cytochrome P450-mediated hydroxylation of the pyridyl ring.[22] It can also be used in the treatment of malignant mesothelioma in companion animals.[23] Since piroxicam is increasingly used in the armamentary of veterinary medicine, there is a need for acute toxicity study of the drug with a view to establishing its toxicity potentials in monogastric animals.

Materials and Methods

A total of 66 animals comprising six female mice, kittens, broilers, local hens, turkeys, ducks, pigeons, guinea pigs, white monkeys, rats, and rabbits weighing 0.025 ± 0.0, 2.2 ± 0.2, 1.9 ± 0.3, 1.3 ± 0.1, 2.3 ± 0.4, 1.5 ± 0.1, 0.3 ± 0.0, 0.5 ± 0.1, 1.4 ± 0.3, 0.3 ± 0.1, and 1.1 ± 0.5 kg, respectively, were used. Piroxicam manufactured by Shanxi Federal Pharmaceutical Co. Ltd. with the batch no 121103 and NAFDAC no 04-6809 was used for the experiment. The manufacturing and expiry dates of the drug are June 2013 and June 2015, respectively. Mice and rats were housed in metal cages, and the remaining species of animals were kept in the Departmental Laboratory of Veterinary Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, Makurdi, Benue State, Nigeria. Water and food were provided ad libitum. Care was provided for the animals as recommended.[24] All institutional and national guidelines for the care and use of laboratory animals were followed.

Preparation of piroxicam solution. Approximately 20 g of piroxicam from capsules was dissolved in 80 mL of distilled water to make 20% solution that was administered orally to all the animals.

Determination of LD_{50}. Six female mice with an average weight of 0.025 ± 0.0 kg were used for the determination of LD_{50}. Each animal was given a single oral dose of piroxicam, with doses chosen to bracket the lethal and surviving doses. Animals were observed for 14 days for onset, nature, severity, and reversibility of toxicity signs as well as the timing of lethality after acute piroxicam exposure. The first, second, third, fourth, fifth, and sixth mice were administered piroxicam at a dose of 103.75, 207.5, 415, 207.5, 415, and 207.5 mg/kg body weight, respectively. But 415 and 103.75 mg/kg were the therapeutic doses selected for rats. The first, second, third, fourth, fifth, and sixth guinea pigs were administered piroxicam orally at a dose of 500, 125, 250, 125, 250, and 62.25 mg/kg, respectively. But the first, second, third, fourth, fifth, and sixth rabbits were administered piroxicam by gavage at a dose of 1000, 415, 1000, 415, 1000, and 415 mg/kg, respectively. However, the first, second, third, fourth, fifth, and sixth cats were administered piroxicam at a dose of 1000, 250, 250, 500, 500, and 125 mg/kg body weight, respectively. Nevertheless, the first, second, third, fourth, fifth, and sixth monkeys were administered piroxicam orally at doses of 1000, 500, 750, 500, 750, and 500 mg/kg, respectively. But the first, second, third, fourth, fifth, and sixth pigeons were administered piroxicam by gavage at doses of 500, 250, 500, 250, 500, and 250 mg/kg, respectively. Whereas the first, second, third, fourth, fifth, and sixth hens were administered piroxicam at doses of 415, 1000, 500, 1000, 415, and 500 mg/kg body weight, respectively. Nevertheless the first, second, third, fourth, fifth, and sixth broilers were consecutively administered piroxicam orally at doses of 415, 207.5, 415, 51.8, 415, and 207.5 mg/kg, respectively. However, the first, second, third, fourth, fifth, and sixth ducks were administered piroxicam at doses of 415, 207.5, 415, 207.5, 415, and 207.5 mg/kg body weight, respectively. The first, second, third, fourth, fifth, and sixth turkeys were administered piroxicam by gavage at doses of 415, 1000, 415, 1000, 415, and 1000 mg/kg, respectively. The therapeutic dose selections were based on the pilot study of piroxicam conducted on mice. LD_{50} of all the species of animals was determined using the method by Saganuwan.[25] After the experiment, the disposed carcasses of turkey, duck and hen administered 1000, 415, and 1000 mg/kg body weight of piroxicam were fed to six puppies. All the animals in the state of moribund were euthanized using formalin.

Determination of neurological effects of piroxicam. Four mice weighing 0.025 ± 0.0 kg were used for the study. The first, second, third, fourth, and fifth mice were administered piroxicam at predetermined a dose of 2000, 1000, 415, and 207.5 mg/kg body weight, respectively. Four rabbits weighing 1.1 ± 0.5 kg were administered piroxicam by gavage. The first, second, third, and fourth rabbits were administered piroxicam orally at a dose of 2000, 1000, 415, and 207.5 mg/kg body weight, respectively. However, three hens with an average weight of 1.3 ± 0.1 kg were used. The first, second, and third hens were administered piroxicam orally at a dose of 2000, 1000, and 415 mg/kg body weight, respectively. Nevertheless, three broilers weighing 1.9 ± 0.3 kg were administered piroxicam by gavage, and the first, second, and third broilers were administered piroxicam orally at a dose of 2000, 1000, and 415 mg/kg body weight, respectively. Turkeys weighing 2.3 ± 0.4 kg were used in this study, and the first, second, and third turkeys were administered piroxicam orally at a dose of

2000, 1000, and 415 mg/kg, respectively. Rationale for using various doses to determine LD_{50} of piroxicam in monogastric animals is because of sensitivity of the species to piroxicam. Mouse is most sensitive.[26] All the animals were observed for extrapyramidal effects, sedation, antimuscarinic effect, and decreased blood pressure. Stethoscope was used to measure the heart rate of the animals, and atropine (0.2 mg/kg) was used to counteract extrapyramidal effects. The quality of extrapyramidal syndrome (torticollis, opisthotonus), sedation, and antimuscarinic and hypotensive effects were recorded. All the animals were taken care according to the recommendations of the Department of Veterinary Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, Makurdi, Nigeria, given the permit no of 0022015.

Statistical analysis. The intensity of observed extrapyramidal effects, sedation, and antimuscarinic and hypotensive effects were assessed qualitatively.[27] Blood pressure below normal was marked as "low intensity". Further decrease in blood pressure by 10 mmHg was considered "moderate intensity", and further decrease of 10 mmHg (ie, 20 mmHg) was marked as "high intensity". The measurements were taken before and 1–3 hours after the administration of piroxicam.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Results

The LD_{50} of mice was 259.4 ± 51.9 mg/kg, the default dose progression was 127.1 (log 2.1), and the confidence interval (CI) was 20%. But at 207.5 mg/kg, mice exhibited various toxicity signs including death and edema of the left forelimb. But at 415 mg/kg, all the mice died. Gross lesions showed gastrointestinal bleeding, congestion of liver, edema of left forelimb, and engorged urinary bladder. The LD_{50} of rat was 259.4 ± 69.6 mg/kg body weight, the default dose progression was 170.5 (log 2.2), and the CI was 26.8%. The observed gross lesions are generalized internal bleeding, and congestion of lung, liver, and heart (Table 1).

The LD_{50} of piroxicam in guinea pig was 218.7 ± 64.1 (mg/kg), the default dose progression was 156.9 mg/kg (log 2.2), and the CI was 29.3%. Paleness of carcass and gastroenteritis were observed. However, the estimated LD_{50} of rabbit was 707.5 ± 130.8 mg/kg with standard deviation (SD) of 320.4 (log 2.5) and CI of 18.5%, rating piroxicam in rabbits as moderately toxic. The observed gross lesions were diarrhea, gastroenteritis, and paleness of carcass. The LD_{50} of piroxicam in cat was estimated to be 437.5 ± 128.1 mg/kg body weight, whereas the default dose progression was 313.7 (log 2.5) and

Table 1. Toxicity pattern of oral piroxicam in monogastric animals.

DOSE (mg/kg)	SURVIVAL STATUS	TOXICITY SIGNS
	Guinea pig	
500	X	Opisthotonus, death after 3 days
125	O	Apparently normal
250	X	Lethargy, off feed, death in 10 days
125	O	Apparently normal
250	O	Apparently normal
62.25	X	Death with prior sign
	Mouse	
103.75	O	Apparently normal
207.5	X	Lethargy, death within 48 hours and edema of left forelimb
415	X	Opisthotonus, jerking of the forelimbs and death in 24 hours
207.5	O	Lethargy, edema of the left forelimb
415	X	Opisthotonus, jerking of the forelimbs, death in 24 hours
207.5	O	Apparently normal
	Rat	
415	X	Opisthotonus, death in 24 hours
103.75	O	Apparently normal
415	X	Opisthotonus, death in 48 hours
103.75	O	Apparently normal
415	X	Opisthotonus, death in 48 hours
103.75	O	Apparently normal
	Rabbit	
1000	X	Opisthotonus, death in 4 days
415	O	Lethargy
1000	X	Opisthotonus, death in 7 days
415	O	Lethargy
1000	O	Lethargy
415	X	Death in 12 days
	Cat	
1000	X	Opisthotonus, death in 24 hours
250	O	Apparently normal
250	O	Apparently normal
500	X	Lethargy, somnolence, death within 3 days
500	X	Somnolence, death within 48 hours
125	O	Apparently normal
	White monkey	
1000	X	Opisthotonus, death
500	O	Lethargy
750	X	Somnolence, death
500	O	Lethargy
750	X	Somnolence, death
900	O	Lethargy

Abbreviations: X, death; O, survival.

the CI was 29.3%. Generalized internal hemorrhage, congestion of liver and lung, and gastroenteritis were observed. The LD_{50} was 733.3 ± 83.3 mg/kg, the default dose progression was 204.1, and the CI was 11.4% for white monkey. Paleness of carcass and gastroenteritis were observed (Table 1).

The LD_{50} of piroxicam in pigeons was 375 ± 55.9 mg/kg, default dose progression was 136.9 mg/kg (log 2.1), and the CI was 14.9%. Grossly, there were generalized internal hemorrhage; congestion of lung, heart, and liver; gastroenteritis; and paleness of carcasses. The LD_{50} was 638.3 ± 115.4 mg/kg with default dose progression of 282.7 mg/kg (log 2.5) and CI of 18.1%, rating piroxicam as moderately toxic in local hens. The observed gross lesions were gastroenteritis, generalized internal hemorrhage, diarrhea, and congestion of lung, liver, and heart (Table 2). The LD_{50} was 285.3 ± 62.5 mg/kg having default dose progression of 153 mg/kg (log 2.1) and CI of 21.9%, rating piroxicam as very toxic in broilers. Paleness of carcass, watery crop content, dryness of pericardium, gastroenteritis, intestinal perforation, and whitish pericardium were observed in broilers. The LD_{50} was 311.3 ± 46.5 mg/kg, the default dose progression was 113.7 mg/kg (log 2.1), and the CI was 14.9% in duck. The gross lesions observed were paleness of carcasses; congestion of lung, liver, and heart; and gastroenteritis. The LD_{50} of oral piroxicam in turkey was 707.5 ± 130.8 mg/kg with the default dose progression of 320.4 mg/kg (log 2.5) and CI of 18.5%, rating piroxicam as moderately toxic in turkeys. The observed gross lesions were anemia, generalized internal bleeding, cheesy lung, and diarrhea (Table 2).

The results of typical antipsychotic effects of piroxicam are shown in Table 3. Mouse administered piroxicam, 2000 mg/kg body weight showed the most intensive extrapyramidal effect manifested by tilted head and neck (opisthotonus). The intensity of the effect decreased as the dose decreased. The animals showed decreased sedation, antimuscarinic effect, and hypotension as the dose decreased. However, at 2000 mg/kg, extrapyramidal effects were very high but sedation, antimuscarinic, and hypotensive effects of piroxicam in rabbits were moderate. At 1000 mg/kg, all the antipsychotic effects were less intensive and below 415 mg/kg only hypotension was observed (Table 3). Hen administered piroxicam, 2000 mg/kg showed intensive extrapyramidal effect (opisthotonus, torticollis), but at 1000 mg/kg the effect was reduced. Sedation, antimuscarinic, and hypotensive effects were absent at 415–103.25 mg/kg body weight. Broiler administered piroxicam, 2000 mg/kg body weight, showed high intensive extrapyramidal effect (opisthotonus, torticollis) and moderate intensive sedation, antimuscarinic, and hypotensive effects. At 1000 mg/kg, the typical extrapyramidal effects of piroxicam were less severe. But between 415 and 103.25 mg/kg, the effects were not observed. Turkeys showed the most serious extrapyramidal syndrome and sedation, antimuscarinic, and hypotensive effects. The extrapyramidal syndrome was most severe at 2000 mg/kg body weight, moderate at 1000 mg/kg. At 415–103.25 mg/kg, all the effects were not observed. However, the extrapyramidal effects were attenuated by atropine at 0.2 mg/kg body weight (Table 3). However, the six puppies fed on the carcasses of turkey, duck, and hen poisoned with piroxicam showed neurological signs such as tremor, salivation, and hyperexcitability.

Table 2. Toxicity pattern of oral piroxicam in domestic birds.

DOSE (mg/kg)	SURVIVAL STATUS	TOXICITY SIGNS
	Pigeon	
500	X	Lethargy, death in 4 days
250	O	Apparently normal
500	X	Lethargy, death in 12 days
250	O	Apparently normal
500	X	Off-feed, death in 7 days
250	O	Apparently normal
	Local hen	
415	O	Lethargy, opisthotonus, death
1000	X	Opisthotonus, torticollis, death
500	X	Lethargy, death
1000	O	Lethargy
415	O	Apparently normal
500	X	Torticollis, death
	Broiler	
415	X	Somnolence, opisthotonus, death in 7 days
207.5	O	Lethargy
415	O	Lethargy, somnolence
51.8	X	Death in 12 days
415	X	Lethargy, opisthotonus, death in 4 days
207.5	O	Apparently
	Duck	
415	X	Opisthotonus, death in 24 hours
207.5	O	Apparently normal
415	X	Somnolence, torticollis, death in 7 days
207.5	O	Apparently normal
415	X	Lethargy, death in 12 days
207.5	O	Apparently normal
	Turkey	
415	O	Apparently normal
1000	X	Torticollis, somnolence, death in 12 hours
415	O	Anorexia. lethargy
1000	X	Opisthotonus, roaring, lethargy, death in 12 hours
415	O	Apparently normal
1000	X	Torticollis, gasping for air, death in 48 hours

Abbreviations: X, death; O, survival.

Table 3. Typical extrapyramidal effects of oral piroxicam in monogastric animals.

DOSE (mg/kg)	EPS	SEDATION	ANT-MUSCARINIC EFFECT	HYPOTENSIVE EFFECT
	Mice			
2000	+++	++	++	++
1000	++	++	++	++
415	+	+	+	+
207.5	+	+	+	0
	Rabbits			
2000	+++	++	++	++
1000	++	+	+	+
415	+	0	0	+
207.5	0	0	0	+
	Local hens			
2000	+++	+++	++	++
1000	++	++	+	+
415	+	0	0	+
	Broilers			
2000	+++	++	++	++
1000	++	++	++	++
415	0	0	0	+
	Turkeys			
2000	+++	+++	+++	+++
1000	++	++	++	++
415	0	0	0	0

Abbreviations: EPS, extrapyramidal symptoms; +++, highest intensity; ++, moderate intensity; +, low intensity; 0, no effect.

Three out of the six puppies died, and the remaining were revived using atropine.

Discussion

The LD_{50} of piroxicam in mice (259.4 ± 51.9 mg/kg), rat (259.4 ± 69.6 mg/kg), guinea pig (218.7 ± 64.1 mg/kg), and cat (437.5 ± 128.1 mg/kg) shows that laboratory animals are very sensitive to acute toxicity of piroxicam. However, the LD_{50} of piroxicam in monkey (733.3 ± 83.3 mg/kg) and rabbit (707.5 ± 130.8 mg/kg) may suggest that piroxicam is moderately toxic in these species. But the LD_{50} of piroxicam in pigeon (375 ± 55.9 mg/kg), hen (638.3 ± 115.4 mg/kg), broiler (285.3 ± 62.5 mg/kg), duck (311.3 ± 46.4 mg/kg), and turkey (707.5 ± 130.8 mg/kg) is suggestive of piroxicam being moderately to seriously toxic in birds. Agents with LD_{50} of 50–500 mg/kg and 500–5000 mg/kg are very toxic and moderately toxic, respectively.[28]

The estimated LD_{50s} of piroxicam in this study disagrees with the report that if the starting dose is greater than the true LD_{50} of the test population, the estimated LD_{50} will tend to be greater than the true LD_{50}.[29] However, it has been reported that LD_{50} is between the doses for the live and the dead animals with reasonable confidence.[30] Another cause of interspecies differences in response to toxicants is the extent of plasma protein binding.[31] Rhesus monkey, rabbit, and guinea pig resemble man in having a relatively high affinity for binding salicylate, a nonsteroidal anti-inflammatory drug (NSAID), similar to piroxicam in pharmacological action. But baboon, dog, rat, and mouse have a low binding capacity.[32] Dissimilarity in intermediary metabolism is among the most frequent causes of differences in susceptibility of various species to toxicants.[33] However, the study outcome is likely to be influenced by the choice of the starting dose level(s), relative to the true LD_{50} value, especially in the case of shallow slope.[34] Therefore, information on toxic effects seen only at dose levels close to a lethal dose will not be obtained.[35] In addition, because small numbers of animals were used, the actual level of confidence was generally not exact.[36] Overall, the random stopping rule in up-and-down procedure (UDP) not only improves the ability of the test to respond to varying underlying conditions but also causes the reported level of confidence and the actual level of confidence to differ slightly.[37] Of all the principles of the three alternatives (fixed dose, acute toxic class, and up-and-down), up-and-down is the most suitable because it gives end point evident toxicity and obeys stopping criteria to limit the number of animals used,[38] using only two to six animals with possible one to three deaths.[25] The improved UDP provides a point estimate

of the LD_{50} and approximates CIs in addition to observing toxicity signs for the substance tested. Acute study performed principally in rodents provides information on the health hazards likely to arise from short-term exposure and are usually an initial step in the evaluation of the toxic characteristics of a substance for both health and environmental effects. The CI is a gage of the capacity of the data collected to give information on the spread of possible response, and it provides plausible bounds on the locations of the LD_{50} values.[29] Actual coverage for the nominal 95% CI will have coverage of at least 90% if the slope is 2–4 or more (σ, 0.25–0.5 or less). For most situations, the coverage will be better than 90% if the slope is 2 or greater. Coverage will be 80% or better if the slope is at least 1. For slopes as low as 0.5, the coverage may be as low as 70%.[39] CIs can be calculated as a function of the sample variance and the number of animals tested to provide bounds on the LD_{50} location and slope.[40] Therefore, there are precision, validity, and reliability of using arithmetic mean as rough estimates of LD_{50}, SD as default dose progression, and standard error of mean as lower and upper boundary.[30]

The neurological signs observed in this study may be due to dopamine released by neurons in the basal ganglia of the brain which is crucial for maintaining normal coordination of movement. Therefore, opisthotonos and torticollis observed in animals administered of piroxicam at a dose of 415–1000 mg/kg body weight may be due to blocking of dopamine receptors. Our findings agree with the report that the loss of dopamine-2 (d_2) receptors is responsible for neurological disorders. In addition, the somnolence showed by turkey administered piroxicam at a dose of 415–1000 mg/kg indicates that piroxicam may cause depression of central nervous system. The observed acute pyramidal effects of piroxicam are similar to those produced by typical antipsychotics used in the treatment of schizophrenia.[41] Opisthotonos is an extrapyramidal effect caused by spasm of the axial muscles and the spinal column, which is seen in severe cerebral palsy and traumatic brain injury, tetanus, kernicterus, meningitis, reduced brain function, severe acute hydrocephalus, transection of the midbrain between the superior colliculus and the inferior colliculus, which results in severing all the corticoreticular fibers. It can be seen in lithium intoxication, haloperidol, metoclopramide, risus sardonicus, strychnine poisoning, heat stroke, decerebrate rigidity, oculogyric crisis, and cerebellar dysfunction. The observed toxic effects such as torticollis and opisthotonus are cerebellar, whereas somnolence and lethargy may be as a result of inhibition of respiratory and cardiac centers in the medulla oblongata.[8] The edema of left forelimb of mice agrees with the report that piroxicam can cause edema.[5] Due to its slow onset of action and delayed attainment of steady state, it is less suited for acute analgesia, and steady-state blood levels are reached in 7–12 days. That could be the reason for death of some animals within 7–12 days after administration. The gastrointestinal lesions such as diarrhea and gastroenteritis observed agree with the report of the European Medicines Agency no longer considers piroxicam a first-line agent,[42] because it produces effects similar to that of fluphenazine (a typical antipsychotic) which cause gastrointestinal lesions in turkeys similar to that of piroxicam.[43]

All the sedation observed in the animals administered piroxicam, 1000–2000 mg/kg shows that at higher doses, piroxicam can cause central nervous system depression. The observed hypotensive effect may suggest that piroxicam has effect on the heart. Therefore, piroxicam may have effect on cerebellum, medulla oblongata, and pons. Extrapyramidal syndrome, sedation, antimuscarinic, and hypotensive effects are typical of antipsychotic drugs.[9] The effects of typical antipsychotic agents are manifested in cerebellum, pons, and medulla[8] and so piroxicam can precipitate congestive heart failure in patients with cardiovascular disease.[42]

A variety of NSAIDs with variable antipyretic effects have been used in dogs and cats, but their use is difficult to justify given the wide range of licensed products.[44] However, high therapeutic doses of piroxicam were translated from human to monogastric animals.[45] Animals feel more pain than humans, since they possess more diffuse neural networks.[46,47] The observed acute pyramidal effects of piroxicam are similar to those produced by phenothiazines used for the treatment of schizophrenia.[22] Dissimilarity in intermediary metabolism is among the most frequent causes of difference in susceptibility of various species to NSAIDs.[33] Single low doses are reasonably effective for treating moderate to severe postoperative pains and can be compared favorably with opioid analgesics such as dextropropoxyphene and tramadol. Few adverse effects were reported, and piroxicam appears to be fairly tolerated in this clinical context. In multiple dosing, the adverse effect profile may be more prominent; therefore, there is a need for quantitative assessment of the efficacy and adverse effects of piroxicam in prolonged dosing regimens.[10]

Secondary poisoning caused by piroxicam agrees with the report that piroxicam caused neurological signs similar to that of typical antipsychotic fluphenazine that caused diarrhea, depression, torticollis, and opisthotonus in turkey.[43]

An amide group of piroxicam is involved in an intramolecular hydrogen bond to the hydroxyl group[48] Derivatives of centrally acting amines in which a primary, secondary, or tertiary amine function has been replaced with a dihydropyridine/pyridinium salt, which are pharmacologically active in vivo, characterized by site-specific and sustained delivery, can penetrate the brain.[49] Brain uptake of piroxicam can be positively correlated with lipid solubility or negatively correlated with hydrogen bonding[50] or as result of damage to the meninges.[51]

Conclusion

The estimated LD_{50} of mice (259.4 ± 51.9 mg/kg), rat (259.9 ± 69.6 mg/kg), guinea pig (218.7 ± 64.1 mg/kg), rabbit (707.5 ± 130.8 mg/kg), cat (437.5 ± 128.1 mg/kg), monkey

(733.3 ± 83.3 mg/kg), pigeon (375 ± 55.9 mg/kg), hen (638.3 ± 115.4 mg/kg), broiler (285.3 ± 62.5 mg/kg), duck (311.3 ± 46.4 mg/kg), and turkey (707.5 ± 130.8 mg/kg) suggested that piroxicam is very to moderately toxic in these species of animals. The observed toxicity signs are extrapyramidal (opisthotonos, torticollis) anemia, roaring, intestinal perforation, and death. Three out of six puppies that fed on the carcasses of poisoned turkeys died, and three puppies were revived by administration of 0.2 mg/kg of atropine sulfates, suggesting cholinergic involvement. Piroxicam has typical extrapyramidal effects at the dose range of 415–2000 mg/kg body weight in laboratory rodents and birds. The effect is characterized by opisthotonus, torticollis, sedation, antimuscarinic, and hypotensive effects. At doses lower than 415 mg/kg body weight, the extrapyramidal effects disappeared in birds. Hence, piroxicam may have an antipsychotic effect similar to that of typical antipsychotics (first-generation antipsychotics). Hence, it may serve as a source for the synthesis of drugs acting on the central nervous system.

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Author Contributions

SAS designed, carried out the study, wrote and approved the manuscript. OAO participated in execution of the study and approved the manuscript.

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