IJPSR (2013), Vol. 4, Issue 10 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 12 May, 2013; received in revised form, 02 August, 2013; accepted, 26 September, 2013; published 01 October, 2013

FORMULATION AND *IN VIVO* EVALUATION OF VETERINARY CHLORPROMAZINE SOLUTIONS FOR INTRAMUSCULAR INJECTION

Nabaa K.A. Al-Hayani and Fouad K. Mohammad*

Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Keywords:

Phenothiazine tranquilizer, Chlorpromazine, Injectable formulation, Sheep, Chicken

Correspondence to Author:

Fouad K. Mohammad

Department of Physiology, Bio-chemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

E-mail: fouadmohammad@yahoo.com

ABSTRACT: The purpose of the study was to present the know-how of preparing intramuscular injection forms of chlorpromazine HCl (CPZ) solutions for use in veterinary medicine. Aqueous solutions of CPZ were prepared aseptically at concentrations of 10 or 25 mg/ml. The CPZ formulations were intended for veterinary use only. The concentrations of CPZ in the solutions were 1 or 2.5% which were formulated mainly with ascorbic acid, benzyl alcohol, sodium chloride, sodium metabisulfite or sodium sulfite and water for injection. The pH values of the solutions were adjusted by HCl or NaOH to be in the range of 5 to 6 before filtration (pore size of 0.2 µm), sterilization and distribution into air tight containers. The aqueous formulations of CPZ were clear, almost colorless to slightly straw-colored solutions, sterile and free from undesirable visible particulate matters. They were according to the requirements of solutions intended for intramuscular injection. Using the prepared formulations, the acute toxicity (median lethal dose, LD50) of CPZ was close to that of a commercial product. Similarly, the effectiveness of CPZ, as determined by the median effective doses (ED50) using the formulations, was close to that of the commercial product and the chicks manifested signs of tranquilization without any adverse effect or death. Further, the preparations were safe and effective when CPZ was injected intramuscularly at the recommended doses experimentally in chicks (10 mg/kg) and sheep (2.2 mg/kg). In conclusion, we introduced simple and applicable know-how of CPZ aqueous formulations (1 or 2.5%) for veterinary use to be administered by deep intramuscular injection.

INTRODUCTION: Chlorpromazine HCl (CPZ), is a dimethylamine derivative of phenothiazine, freely soluble in water [1] and it is used as an antipsychotic drug in human medicine [2, 3] and as a tranquilizer, antiemetic and a preanesthetic in veterinary practice [4, 5].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.4(10).3877-83

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.4(10).3877-83

The mechanism of the tranquilizing action of CPZ is related to antagonism of dopaminergic receptors in the central nervous system [6, 7].

The recommended therapeutic doses of CPZ in dogs, cats, cattle and sheep are usually 0.5, 0.3, 1.1 and 2.2 mg/kg body weight, respectively, given by deep intramuscular injection [4, 5, 8].

Various commercial preparations of CPZ are available for use in human medicine [2]. None is approved as a veterinary formulation [4, 9]. However, CPZ is legally prescribed and used in animals on the basis of an extra-label use of drug [7, 9].

The deep intramuscular injection is the preferred route of CPZ administration in animals because of the suitability of the method and for obtaining a rapid response, usually within 20 minutes [4-8], and avoiding mostly the first pass metabolism by the liver [1, 3, 7]. The blood level of CPZ usually peaks within 2 h of intramuscular injection of the drug [1, 3, 7]. The injectable formulations of CPZ are sterile aqueous solutions available at concentrations of 10 or 25 mg/ml [2, 10]. The formulations of CPZ may contain ascorbic acid, benzyl alcohol and sulfites as preservatives or antioxidants and sodium chloride to adjust for osmolality with the pH ranging between 3.5 and 6 [2, 10].

The purpose of the present study was to develop and present the know-how of potential injectable veterinary formulations of CPZ.

MATERIALS AND METHODS:

Chemicals: The chemicals used were chlorpromazine HCl, sodium chloride, benzyl alcohol, ascorbic acid, sodium sulfite, sodium metabisulfite and water for injection. They were kindly donated by the State Company for Drug and Medical Appliances-Ninevah (Mosul, Iraq). The chemicals were also according to specifications of BP (2012) [11].

Formulation: The chemical contents and their amounts for four injectable veterinary formulations of CPZ are presented in **table 1**. The contents and their amounts were based on our preliminary formulation experiments as well as on the literature [2, 10, 12]. For all the formulations, under aseptic conditions, we first dissolved CPZ in about 800 ml water for injection with continuous stirring. Thereafter, other solid ingredients were added respectively to the CPZ solution, followed by benzyl alcohol when required, with continuous stirring until clear solutions were obtained.

TABLE 1: CONTENTS AND THEIR AMOUNTS FOR INJECTABLE VETERINARY FORMULATIONS OF CHLORPROMAZINE HCl

Formulations	Ingredients	Amount/L
A	Chlorpromazine HCl	10 g
	Ascorbic acid	2 g
	Water for injection to make	1000 ml
B	Chlorpromazine HCl	25 g
	Benzyl alcohol	15 g
	Water for injection to make	1000 ml
C	Chlorpromazine HCl	25 g
	Ascorbic acid	2 g
	Sodium metabisulfite	1 g
	Sodium sulfite	1 g
	Sodium chloride	6 g
	Water for injection to make	1000 ml
D	Chlorpromazine HCl	25 g
	Ascorbic acid	2 g
	Sodium metabisulfite	1 g
	Sodium chloride	1 g
	Benzyl alcohol	20 g
	Water for injection to make	1000 ml

When necessary, we adjusted the pH to a range of 5 to 6 with 10% HCl or 4% NaOH, and thereafter the volume was completed to 1000 ml with water for injection to obtain 1% (A) or 2.5% (B, C and D) solutions of CPZ (Table 1). The final formulations were filtered through pore size of 0.2 µm membrane filters [13], and then distributed into 25 dark amber-colored glass vials or ampoules. We sealed the ampoules and capped the vials and sealed them tightly. Furthermore, the CPZ containers were subjected to heat sterilization by autoclaving [12, 13].

Product evaluation: We examined the injectable 1 or 2.5% aqueous solutions of CPZ formulations as follows: measuring the pH using a pH meter (Hanna Instruments, Romania), sterility test for bacterial (blood and brain-heart agar) and fungal (sabouraud agar) contaminations [14, 15], visual inspection to record the color and any particulate matters that might contaminate the formulations as well as determining the osmolality by freezing point depression osmometer (Osmomat 030 GmBH, Germany).

We determined the concentration of the active ingredient CPZ in the formulations spectrophotometrically [16] with some modifications. An aliquot of 0.1 ml of CPZ solution was diluted to 200 ml with 0.1 N sulfuric acid. Two ml of 50% sulfuric acid was added to 4 ml of the diluted CPZ sample, mixed well and then 0.2 ml of 2% ferric nitrate solution in 1 N sulfuric acid was added.

The absorbance of the developed color was measured by a spectrophotometer (Jenway 6405, Wagteah International, U.K.) at 530 nm against a water blank. The standard curve of CPZ ranged between 10 to 80 µg/4 ml of 0.1 N sulfuric acid.

Determination of acute median lethal dose (LD50) of CPZ in chicks: For safety assessment, each formulation as well as a commercial one was used to determine the acute (24 h) LD50 of CPZ given intramuscularly (i.m.) in 7-14 day-old broiler chicks of both sexes by the up-and-down method [17]. After the CPZ injection, each chick was observed for one h for the appearance of signs of poisoning (usually depression in nature) and then the 24 h lethality was recorded. The initial dose of CPZ was 200 mg/kg, i.m.; we used only five to seven chicks for each formulation.

Determination of median effective tranquilizing dose (ED50) of CPZ in chicks: We determined, by the up-and-down method [17], the ED50 of CPZ in each formulation as well as in the commercial one for the induction of a state of tranquilization and sedation in 7-14 day-old broiler chicks of both sexes [18, 19].

The initial dose of CPZ was 5 mg/kg, i.m.; we used only six to eight chicks for each formulation.

Tranquilizing effect of CPZ in chicks and sheep: Using the four formulations and the commercial one, the tranquilizing effect of CPZ was examined in 7-14 day-old broiler chicks given the drug at the dose rate of 10 mg/kg, i.m. [20]. In adult sheep (50-70 kg) of both sexes, CPZ was administered at the dose rate of 2.2 mg/kg, i.m. [5] using the four formulations we prepared.

After the CPZ injection, the latency of onset of tranquilization and its duration were recorded in chicks and sheep. The signs of tranquilization were reduced motility or immobility, closing eyelids and drooping of the head [18, 19, 21].

Determination of alanine aminotranferase (ALT) and creatine phosphokinase (CPK) activities: In the sheep, blood was obtained from the jugular vein into heparinized test tubes one day before the injection (baseline) and soon after the end of the tranquilizing effect of CPZ (2.2 mg/kg, i.m.) for measurement of ALT and CPK activities using commercially available kits (Biolab, France). The ALT and CPK are useful for monitoring liver and skeletal muscle damages, respectively [22].

***In vitro* erythrocyte hemolysis test:** Aliquots (0.1 ml) of sheep erythrocytes suspended in normal saline solutions were added to one ml of 20 to 100% concentrations of the four formulations of CPZ in normal saline solution. The mixture was incubated at room temperature for 20 min and the percentage of hemolysis was calculated after determination of the hemoglobin content of the supernatant spectrophotometrically using a commercial kit (Biolab, France) as described before [23, 24].

Statistical analyses: Data as multiple means (tranquilizing effects in chicks and sheep) were statistically analyzed by one way analysis of variance followed by Tukey's multiple range test, whereas the data of enzyme activities were statistically analyzed by paired Student's-t-test [25], utilizing the Past Statistics Package (http://folk.uio.no/ohammer/past/index.html). The accepted level of statistical significance was at $p < 0.05$.

Approval of the experiments: The Scientific and Ethics Committee of the College of Veterinary Medicine at the University of Mosul (Iraq) has approved the present experimental protocols.

All experiments complied with the University of Mosul regulations and ethics regarding animal use, and proper attention and care have been given to the animals used in the study.

RESULTS: The four formulations of CPZ as prepared were almost colorless to slightly straw-colored solutions, sterile and free from undesirable visible particulate matters, with pH values between 5 to 6 (**Table 2**). The concentration of CPZ in formulation A was 88% and it ranged between 90 to 92% in the other formulations (Table 2). The osmolalities of formulations A and B were lower than those of C and D counterparts (0.011 and

0.096 vs 212 and 0.198 Osmol/kg), respectively (Table 2).

The LD50 values of CPZ from the four formulations ranged between 175 to 236 mg/kg, i.m., whereas that of the commercial one was 218 mg/kg, i.m. (**Table 3**). The signs of poisoning appeared in the chicks within two to 15 min of drug administration, and consisted of depression, closed eyelids, ruffled feather and recumbency; few birds manifested tremors, diarrhea or salivation before death. The signs of poisoning induced by the four formulations and the commercial one were not qualitatively different.

Further, the ED50 of CPZ in the four formulations ranged between 1.8 to 2.9 mg/kg, i.m. and that of the commercial one was 2.6 mg/kg, i.m. (Table 3). All the birds showed signs of sedation and tranquilization within three to ten min of drug administration, and they were characterized by drooping of the head, closed eyelids, reduced motility and stress calls, drooping of the wings and recumbency. No death occurred during this experiment.

Intramuscular injection of CPZ at 10 mg/kg using the four formulations was effective in producing sedation and tranquilization in chicks within 12.3 to 18 min for 7.3 to 9.7 in a manner comparable to the commercial preparation (**Table 4).** The latency to onset of tranquilization and its duration were not significantly different among the four formulations and also when compared with those of the commercial one (Table 4). Similarly, CPZ was also effective in producing tranquilization in sheep, when injected at 2.2 mg/kg, i.m., within 15 to 20 min for 33 to 43.8 min (**Table 5**).

The latency to onset of tranquilization and its duration were not significantly different among the four formulations (Table 5). All the chicks and sheep recovered smoothly from the trqnquilizing effect of CPZ and none suffered from unexpected side effects or death.

Intramuscular injection of CPZ using the four formulations in sheep did not significantly change ALT and CPK activities when compared with pretreatment values (data not shown). The four formulations of CPZ induced marked *in vitro* hemolysis of sheep erythrocytes at concentrations as low as 20% of the preparations (**Table 6**). Formulation A produced hemolysis by 73 and 62% at the concentrations of 20 and 40% in normal saline solution, respectively and by 100% at higher concentrations of the formulation (60-100%), whereas formulations B, C and D caused 100% hemolysis at all levels (20 to 100%) of the tested concentrations (Table 6).

TABLE 2: CHARACTERISTICS OF VETERINARY CHLORPROMAZINE FORMULATIONS

Variable	Formulation			
	A	B	C	D
Chlorpromazine HCl concentration (%)	1	2.5	2.5	2.5
Color	colorless to slightly straw-colored	colorless to slightly straw-colored	colorless to slightly straw-colored	colorless to slightly straw-colored
Sterility test	sterile	sterile	sterile	sterile
Clarity	clear	clear	clear	clear
Visible particulate matter	none	none	none	none
pH	5.7	5.8	5.7	5.8
Chlorpromazine assay (%)	88	92	92	90
Osmolality (Osmol/kg)	0.011	0.096	0.212	0.198

TABLE 3: THE ACUTE 24 HOUR MEDIAN LETHAL DOSES (LD_{50}) AND MEDIAN EFFECTIVE DOSES (ED_{50}) OF CHLORPROMAZINE USING THE PREPARED FORMULATIONS AND A COMMERCIAL ONE IN 7-14 DAY-OLD BROILER CHICKS AFTER INTRAMUSCULAR INJECTION

Variable	Formulation				
	A	B	C	D	Commercial
LD50 (mg/kg)	208	236	236	175	217
Range of latency to onset of poisoning signs (min)	2-10	4-10	5-15	5-15	5-15
ED50 (mg/kg)	1.9	2.9	1.8	1.8	2.6
Range of latency to onset of tranquilization signs (min)	4-10	4-8	5-8	5-10	3-7
Range of duration of tranquilization (min)	5-9	5-10	4-9	3-6	3-5

TABLE 4: TRANQUILIZING EFFECT OF CHLORPROMAZINE USING THE PREPARED FORMULATIONS AND A COMMERCIAL ONE IN 7-14 DAY-OLD BROILER CHICKS AFTER INTRAMUSCULAR INJECTION AT 10 mg/kg BODY WEIGHT

Formulation	Latency to onset of tranquilization (min)	Duration of tranquilization (min)
A	12.7 ± 1.2	8.3 ± 1.2
B	14.7 ± 1.5	7.3 ± 1.2
C	18.0 ± 1.0	8.7 ± 1.0
D	12.3 ± 1.5	9.7 ± 1.0
Commercial	14.7 ± 1.5	9.0 ± 1.0

Values are mean ± SE of 3 chicks/group.

TABLE 5: TRANQUILIZING EFFECT OF CHLORPROMAZINE USING THE PREPARED FORMULATIONS IN SHEEP AFTER INTRAMUSCULAR INJECTION at 2.2 mg/kg BODY WEIGHT

Formulation	Latency to onset of tranquilization (min)	Duration of tranquilization (min)
A	16.8 ± 3.8	33.0 ± 5.2
B	20.0 ± 4.1	33.8 ± 5.5
C	18.8 ± 4.3	43.8 ± 2.4
D	15.0 ± 2.9	33.8 ± 6.9

Values are mean ± SE of 4 sheep/group.

TABLE 6: PERCENTAGES OF *IN VITRO* HEMOLYSIS OF SHEEP ERYTHROCYTE SUSPENSIONS BY FOUR INJECTABLE VETERINARY FORMULATIONS OF CHLORPROMAZINE

% formulation	Chlorpromazine formulation			
	A (1%)	B (2.5%)	C (2.5%)	D (2.5%)
20	73	100	100	100
40	62	100	100	100
60	100	100	100	100
80	100	100	100	100
100	100	100	100	100

DISCUSSION: The four CPZ formulations were found to be sterile, clear injectable solutions, free from visually detectable particulate matters and complied with the requirements of aqueous solutions intended for parenteral administration [11, 13]. The specifications of the four veterinary formulations of CPZ depended mainly on the high solubility of the active ingredient in the aqueous phase that can be suitably sterilized by filtration/and or autoclaving [1, 2]. Sodium chloride was added in formulations C and D to help in adjusting the tonicity of the solution (Table 2) so that they would reduce possible tissue damage after intramuscular injection.

However, this additive did not appear to be necessary in this case, since *in vivo* experiments in the sheep demonstrated that deep intramuscular injection of CPZ using the four formulations did not produce muscular or liver damages. This is because post-injection plasma CPK and ALT activities were not significantly changed compared to respective pretreatment values. Increased ALT and CPK activities are indicatives of liver and musclular damages, respectively [22].

Several studies have suggested using *in vitro* erythrocyte hemolysis test as a mean of predicting tissue damaging effect of pharmaceutical preparations intended for intramuscular administration [23, 24, 26].

However, based on our findings in the present study, the *in vitro* erythrocyte hemolysis test should not be applied to evaluate potential tissue irritating ability of CPZ injectable preparations, since the drug has inherent activity of causing *in vitro* osmotic hemolysis of erythrocytes by binding to cell membrane proteins [27-29]. In support of this notion, we found that the four formulations of CPZ induced marked in vitro hemolysis of sheep erythrocytes at concentrations as low as 20% of the preparations (Table 6).

In accordance with our findings, CPZ was found to cause *in vitro* erythrocyte hemolysis but with muscular toxicity in rabbits [26]. The discrepancy regarding the CPK activity between the study in rabbits [26] and the present study could be attributed to species variation, type of the formulation used and dosage regimen.

We used benzyl alcohol in the formulations as a preservative and sodium metabisulfite as well as sodium sulfite for their antioxidant and preservative properties [13, 14]. Benzyl alcohol is safe upto 5% in pharmaceutical formulations [30], but it was reported to cause erythrocyte hemolysis *in vitro* [31].

Therefore, it is possible that benzyl alcohol might have contributed to the *in vitro* hemolysis of sheep erythrocytes seen with the products B and D in the present study.

The experiments we conducted in chicks revealed that the acute toxicity (LD_{50}) of CPZ was close to that of the commercial product and it was not more than those reported in chickens (160 mg/kg, intraperitoneally; 28 mg/kg, intravenously), rats (62 mg/kg, intraperitoneally) or in mice (420 mg/kg, subcutaneously; 92.2-115 mg/kg, intraperitoneally) [1, 32], taking into account the relative differences according to routes of drug administration.

The ED_{50}s of CPZ using the four formulations were close to that of the commercial product and the chicks manifested signs of tranquilization without any adverse effect or death, and these values were also comparably to the doses of CPZ reported to be effectively used in chicks [20, 33, 34].

Further *in vivo* experiments also demonstrated the clinical effectiveness of the four formulations in tranquilizing the chicks at 10 mg/kg, i.m. and sheep at 2.2 mg/kg, i.m. without untoward effects. Similar dosages were reported to be clinically effective in chicks [20] and sheep [5]. CPZ is clinically used, mainly for its tranquilizing effect, in various animal species, except the horses [4-8].

The prepared CPZ formulations should be protected from light and stored in air tight amber-colored glass vials, below 25 °C [1, 2, 32]. The label of the present CPZ formulations is expected to state the drug concentration, route of administration, the dosages according to animal species, storing condition and the expiry date as well as a warning that is a veterinary product not to be used in humans only.

CONCLUSION: In conclusion, we introduced simple and applicable know-how of CPZ aqueous formulations (1 or 2.5%) for veterinary use to be administered by deep intramuscular injection.

ACKNOWLEDGMENTS: This report represents a portion of a thesis to be submitted by the first author to the University of Mosul, Iraq as partial fulfillment of the requirements of an MSc degree in Veterinary Pharmacology and Toxicology. This study was supported by the College of Veterinary Medicine, University of Mosul, Iraq.

REFERENCES:

1. IPCS INCHEM: Chlorpromazine. [Cited April 20, 2013]; Available from http://www.inchem.org/documents/pims/pharm/chlorpro.htm
2. Sweetman SC, editor: Martidale. The Complete Drug Reference. Pharmaceutical Press, London, UK, 2006: 977.
3. Brunton L, Parker K, Blumenthal D and Buxton L: Goodman and Gilman's Manual of Pharmacology and Therapeutics. McGraw-Hill Co., Inc., New York, USA, 2008: 299-318.
4. Plumb DC: Veterinary Drug Handbook. Iowa State Press, New York, USA, 2002: 64-67.
5. Papich MG: Saunders Handbook of Veterinary Drugs. Elsevier-Saunders, St. Louis, MO, USA, 3rd Edition 2011: 148-149.
6. Crowell-Davis SL and Murray T: Veterinary Psychopharmacology. Blackwell Publishing, Ames, Iowa, USA, 2006: 154-155.
7. Posner LP and Burn P: Sedative agents: tranquilizers, alpha-2 agents, and related agents. In: Riviera JE and Papich MG. Veterinary Pharmacology and Therapeutics. Wiley-Blackwell, Ames, Iowa, USA, 2009: 337-380.
8. Haskell SRR and Anttila TA: Small Ruminant Clinical Diagnosis and Therapy. College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota, USA, 2001.
9. Ruben D: Chlorpromazine (Thorazine®). [Cited April 20, 2013]; Available from http://www.petplace.com/drug-library/chlorpromazine-thorazine/page1.aspx
10. West-Ward Pharmaceutical Corporation: Chlorpromazine injection. [Cited April 21, 2013]; Available from http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?id=77883
11. BP: British Pharmacopoeia. The Stationary Office, UK, 2012.
12. Niazi SK: Handbook of Pharmaceutical Manufacturing Formulations: Sterile Products. Vol. 6. CRC Press, Boca Raton, USA, 2004.
13. Allen LV Jr and Popovich NG and Ansel HC: Pharmaceutical Dosage Forms and Drug Delivery Systems. Lippincott Williams and Wilkins, Philadelphia, USA, 8th Edition 2005: 460.
14. Blodingers J: Formulation of Veterinary Dosage Form. Marcel Dekker, Inc., New York, USA, 1983.
15. Brooks GF, Butel JS and Morse SA: Medical Microbiology. Lange Medical Books, New York, USA, 2001.
16. Leach H and Crimmin WRC: The colorimetric estimation of 3-chlorpromazine in biological fluids. Journal of Clinical Pathology 1956; 9: 164-165.
17. Dixon WJ. Efficient analysis of experimental observations. Annual Review of Pharmacology and Toxicology 1980; 20: 441-462.
18. Al-Zubaidy MHI and Mohammad FK. Metoclopramide-induced central nervous system depression in chickens. BMC Vet Res. [online]. 2005 [cited April 22, 2013]; 1:6, Available from http://www.biomedcentral.com/1746-6148/1/6

19. Mohammad FK, Al-Zubaidy MHI and Alias AS. Sedative and hypnotic effects of combined administration of metoclopramide and ketamine in chickens. Lab Animal 2007; 36: 35-39.
20. Maser JD, Gallup GG, Hicks LE and Edson PH: Chlorpromazine dosage and duration of tonic immobility: biphasic effects. Pharmacology Biochemistry and Behavior 1974; 2: 119-121.
21. Habib S, Das BC, Islam MN, Hossain MK and Ahmed MF: A comparison of xylazine, diazepam, chlorpromazine and promazine in relation to certain clinical and hematological parameters of indigenous sheep (*Ovis aries*). Pakistan Journal of Biological Sciences 2002; 5: 484-488.
22. Kerr MG: Veterinary Laboratory Medicine. Blackwell Science Ltd., London, UK, 2nd Edition 2002.
23. Surber C and Dubach UC: Tests for local toxicity of intramuscular drug preparations. Comparison of *in vivo* and *in vitro* findings. Drug Research 1989; 39: 1586-1589.
24. Mohammad FK, Al-Baggou', BKh, Hachem IM and Said MO: Tests for local irritation of veterinary intramuscular antibacterial preparations. Iraqi Journal of Veterinary Sciences 2002; 16: 101-105.
25. Petrie A and Watson P: Statistics for Veterinary and Animal Sciences. Blackwell Science, Oxford, UK, 1999.
26. Højelse Fl, Svendsen O and Bagdon RE: Tests for local toxicity of intramuscular drug preparations. Comparison of *in vitro* and *in vivo* methods. Archives of Toxicology 1986; 8: 474-475.
27. van Steveninck J, Ghosund WK and Booij HL: The influence of chlorpromazine on the osmotic fragility of erythrocytes. Biochemical Pharmacology 1967; 16: 837-841.
28. Mao TS and Noval JJ: Binding of phenothiazines to proteins-measurement of binding based on the inhibition of the hemolytic activity of phenothiazines on sheep red blood cells. Biochemical Pharmacology 1973; 22: 2497-2500.
29. Thompson AA, Cornelius AS, Asakura T and Horiuchi K: Comparative studies of phenothiazine derivatives for their effects on swelling of normal and sickle erythrocytes. General Pharmacology 1993; 24: 999-1006.
30. Nair B: Final report on the safety assessment of benzyl alcohol, benzoic acid, and sodium benzoate. International Journal of Toxicology 2001; 20 Supplement 3: 23-50.
31. Ogiso T, Iwaki M and Yamamoto M: Hemolysis induced by benzyl alcohol and effect of the alcohol on erythrocyte membrane. Chemical and Pharmaceutical Bulletin (Tokyo) 1983; 31: 2404-2415.
32. MDI Information Systems, Incorporation: Material safety data sheet. Chlorpromazine hydrochloride. MDI Information Systems, Inc., USA, 2001.
33. Al-Zubaidy MHI and Mohammad FK. Effects of acute manganese neurotoxicity in young chicks. Archives of Industrial Hygiene and Toxicology 2013; 64: 69-76.
34. Barnes CD and Eltherington LG. Drug Dosage in Laboratory Animals. University of California Press, Berkeley, CA, USA, 1973.

How to cite this article:
Al-Hayani NKA and Mohammad FK: Formulation and *in vivo* evaluation of veterinary Chlorpromazine solutions for intramuscular injection. *Int J Pharm Sci Res* 2013; 4(10): 3877-83. doi: 10.13040/IJPSR. 0975-8232.4(10).3877-83