# Preparation and Evaluation of Veterinary 0.1% Injectable Solution of Atropine Sulphate

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Abstract

This study introduces the know-how of preparing a multiple injection form atropine sulphate solution. An injectable aqueous solution of atropine sulphate at a concentration of 0.1%. was prepared under aseptic conditions in dark glass bottles each containing 50 ml. The preparation was intended for animal use only. It contained 1g atropine sulphate, 9 g sodium chloride as a normal saline, benzyl alcohol 15 ml as a preservative and water for injection up to 1000 ml. The pH of the solution was adjusted to 4.2 (range 3.0-6.5). The preparation of 0.1% atropine sulphate solution was clear colorless solution free from undesired particles. It complied with the requirements for injectable solutions. Further, the preparation was safe when used under laboratory conditions in chicks, rats and donkeys. It was also effective in preventing dichlorvos (an organophosphate insecticide)-induced poisoning in chicks in a manner comparable to a commercial preparation of 0.1% atropine sulphate. In conclusion, the know-how of a preparation of 0.1% atropine sulphate solution is presented for veterinary use.
Keywords: Atropine sulphate, Injectable formulation, Organophosphate.

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## Introduction

Atropine is a naturally occurring tertiary amine isolated from the *Atropa belladonna* plant (deadly nightshade); it is a competitive acetylcholine antagonist at the muscarinic receptors (Brunton *et al.*, 2008). Atropine sulphate is odorless, colorless or white crystalline bitter powder, and it is highly soluble in water (Parfitt, 1999; McKeown, 2002). It is mostly used in veterinary practice as a preanesthetic agent to achieve balanced anesthesia and as a specific antidote against organophosphate and carbamate insecticides (Plumb, 2002) which cause parasympathetic effects as a result of acetylcholinesterase inhibition leading to acetylcholine accumulation at the nerve endings (Marrs and Vale, 2006). Atropine is also useful in treating diarrhea and muscarine poisoning following ingestion of certain fungi (Plumb, 2002; Bishop, 2005). Furthermore, the drug has some ophthalmic uses (Bishop, 2005; Brunton *et al.*, 2008).

The recommended therapeutic dose of atropine in animals is usually between 0.15-0.3 mg/kg, intramuscularly (i.m.) as a preanesthetic agent and in case of organophosphate poisoning it is 0.5 mg/kg, (1/4 intravenously (i.v.), rest subcutaneously (s.c.) or intramuscularly), which could be repeated at 3-4 hours intervals for 1-2 days (Plumb, 2002; Bishop, 2005). Higher doses of the drug can be used when needed in cases of organophosphate poisoning (Osweiler, 1996).

Many commercial preparations of the injectable forms of atropine sulphate are available for use in veterinary practice (Entriken, 2001;

Bishop, 2005). These are sterile solutions in normal saline or water for injection from several manufacturers (Entriken, 2001; Bishop, 2005). Atropine sulphate solution with a pH range of 3 to 6.5 (McKeown, 2002; USP, 2002) is usually available in concentrations of 0.1-0.5 mg/ml as a single injectable or multiple dosage forms (Entriken, 2001; Bishop, 2005). The preservatives benzyl alcohol, parabens and sulfites, may be found in such injectable products of atropine sulphate (Entriken, 2001; McKeown, 2002; Bishop, 2005).

The veterinary injectable dosage forms of atropine sulphate in the current market place of Iraq are rather imported. The amount of atropine sulphate in these preparations is specified (e.g. 0.1%). However, other ingredients used in the preparation of atropine sulphate solutions are not known quantitatively. The purpose of the present report was to introduce the know-how of a successful approach to prepare an injectable multiple dosage form of atropine sulphate suitable for use in animals.

## Materials and Methods

The chemicals used were atropine sulphate, sodium chloride, benzyl alcohol and water for injection. They were according to specifications of BP Vet (2000). The ingredients and their amounts of the injectable multiple dosage form of 0.1% atropine sulphate are shown in table 1.

Table-1. The ingredients and their amounts of the veterinary injectable solution of 0.1% atropine sulphate

| Ingredients | Amount/1000 ml |
|---|---|
| Atropine sulphate | 1 g |
| Sodium chloride | 9 g |
| Benzyl alcohol | 15 ml |
| Water for injection to make | 1000 ml |

Atropine sulphate and sodium chloride were dissolved in about 900 ml water for injection with continuous stirring. Then, benzyl alcohol was added to the solution with continuous stirring until a clear solution was obtained. When necessary, we adjusted the pH to 4.2 (range 3.0-6.5) with sulfuric acid or sodium hydroxide solutions and thereafter the volume was completed to 1000 ml with water for injection. The final solution of atropine sulphate was sterilized by filtration with pore size of 0.2 µm (Ansel et al., 1999; BP Vet 2000; USP, 2002), and then distributed into 50 ml amber-colored glass bottles . We capped the bottles and sealed them tightly.

The injectable 0.1% solution of atropine sulphate was subjected to various examinations that included the pH of the preparation using a pH meter (Hanna Instruments, Romania), sterility test on blood agar and brain-heart agar for bacteria and sabouraud agar for fungi (Blodingers, 1983; Brooks et al., 2001), visual inspection to determine the color and any macro contaminant and determination of the concentration of the active ingredient atropine sulphate (this assay and additional ones were done by Syphco Co. for Drugs and Chemicals, Damascus, Syria).

For safety evaluation of the product, the preparation of 0.1% atropine sulphate was injected in chicks at 2 mg/kg, s.c. (n=10), donkeys as a preanesthetic at 0.02 mg/kg, i.v. (n=5), and rats at 2 mg/kg, intraperitoneally (i.p.) (n=10). The animals were monitored for any physical unexpected side effects or lethality. To further document the safety and effectiveness of the prepared atropine sulphate solution (0.1%) in comparison with a commercial preparation of 0.1% atropine sulphate solution, an experiment was conducted to antagonize poisoning induced by the organophosphate insecticide dichlorvos in chicks. Atropine is the standard antidote against organophosphate poisoning in animals including the avian species (Osweiler, 1996; McKeown, 2002; Wilson, 2005). For this experiment, mixed breed broiler chicks of either sex (10-15 days old) were used. They were maintained at a temperature of 30-34 $^{O}$C with constant lighting and floor litter consisted of wood shavings; water and feed were given ad libitum. Commercial insecticidal concentrate solution of dichlorvos (55%, SAFA DDVP55EC, Kalite Yonetim, Turkey) was further diluted in distilled water to obtain the desired concentration for oral dosing by a gavage needle in a volume of 5 ml/kg body weight.

Chicks were treated i.p. with the physiological saline solution at 5 ml/kg (the control, n=8) or

Table-2. Demonstration of the antidotal effect of the prepared 0.1% atropine sulphate solution (2 mg/kg, i.p.) in chicks intoxicated with dichlorvos (25 mg/kg, orally)

| Treatment | Latency to onset of signs of poisoning (seconds) | Latency to onset of death (minutes) | Percentage of death (2 & 24 h) |
|---|---|---|---|
| Physiological saline (control) | 36 ± 6 | 94 ± 10 | 100 |
| Atropine sulphate (prepared) | 153 ± 38* | | 0* |
| Atropine sulphate (commercial) | 50 ± 3* | | 0* |

Values are mean ± SE of 7-9 chicks /group.
* Significantly different from the saline-treated control group, $p < 0.05$.

with the prepared 0.1% atropine sulphate at 2 mg/kg (n=9) or with a commercial preparation of 0.1% atropine sulphate at 2 mg/kg (n=7). This dosage of atropine is supposed to be effective in antagonizing organophosphate poisoning (Osweiler, 1996; McKeown, 2002). Fifteen minutes after the injections, all the chicks were dosed orally with dichlorvos (active ingredient) at 25 mg/kg of body weight to induce organophosphate poisoning (Mohammad et al., 2008). The dose of dichlorvos was determined in a preliminary experiment on chicks, and this dose was found to induce death in 80 to 100% of the dosed chicks within 24 h. After the dichlorvos dosing, the chicks were observed for the occurrence of the signs of organophosphate poisoning that are characteristic of cholinergic toxicity (Mohammad *et al*., 2008). The signs included salivation, lacrimation, gasping and convulsion. The 2-h and 24-h lethalities were also recorded. The latencies to onset of any sign of poisoning and death were recorded within 2 h.

Data as multiple means were statistically analyzed by one way analysis of variance followed by the least significant difference test (Petrie and Watson, 1999). The frequency data were subjected to the Fisher's exact probability test (Runyon, 1977). The accepted level of statistical significance was at $p < 0.05$.

The Scientific and Ethics Committee of the College of Veterinary Medicine at the University of Mosul (Iraq) has approved the present study, including the toxicity experiment in chicks. All experiments complied with our institutional regulations and ethics addressing animal use, and proper attention and care have been given to the animals used in the study.

## Results

The injectable dosage form of 0.1% atropine sulphate complied with the requirements for parenteral solutions. The content of atropine sulphate complied with the requirements of this preparation with a range of 93-100%. The prepared dosage form of 0.1% atropine sulphate was clear colorless solution with a pH value of 4.2 (range 3-6.5); it was sterile, free from undesired particles. It appeared to be safe in chicks, rats and donkeys and none of the treated animals showed undesirable side effects or death.

Dichlorvos at 25 mg/kg, orally produced signs of cholinergic toxicosis in the chicks, and these included salivation, lacrimation, gasping and convulsions within 2 h. The 2-h as and 24-h lethalities were 100% in the saline treated control group (Table 2). Atropine sulphate at 2 mg/kg, i.p. as the prepared or the commercial 0.1% solutions given 15 minutes before dichlorvos dosing variably decreased the occurrence of toxic manifestations in the chicks and both of the preparations significantly decreased the latency to onset of signs of organophosphate poisoning and prevented lethality in chicks by 100% as judged 24 h after the dichlorvos dosing (Table 2).

## Discussion

The prepared injectable dosage form of 0.1% atropine sulphate was in compliance with the requirements of parenteral solutions intended for injection (Ansel *et al.*, 1999). The content of atropine sulphate in the prepared samples was more than 93%; the solution was clear, colorless, sterile and free from particles. The specifications of the prepared solution were also well within those required by BP Vet ( 2000) and USP (2002). Atropine sulphate is highly soluble in water, and sodium chloride is added to help keep the tonicity of the preparation without causing tissue damage

after injections (Parfitt, 1999; McKeown, 2002). Benzyl alcohol is used in the preparation as a preservative since it is required in preparations intended for use as multiple dosage forms (Blodingers, 1983; Ansel *et al.*, 1999). Others have used parabens and sulfites in injectable products of atropine sulphate (McEvoy, 2002). Further, the 0.1% preparation of atropine sulphate in the present study was tried experimentally at therapeutic doses in three animal species: chicks, rats and donkeys, and it was found to be safe without producing adverse effects in these animals. Even at a high dose (2 mg/kg), the prepared atropine sulphate solution was therapeutically similar to a commercial preparation of the drug in antagonizing organophosphate poisoning caused by dichlorvos in chicks. This insecticide inhibits acetylcholinesterase activity leading to acetylcholine accumulation at nerve endings and subsequently causing parasympathetic overstimulation (Marrs and Vale, 2006). Our result on the use of atropine against organophosphate poisoning further demonstrate that the prepared solution is as effective as the commercial preparation and conform to the expected efficacy of such a preparation (McEvoy, 2002; McKeown, 2002).

The prepared atropine sulphate solution should be protected from light and stored in multiple-dose air tight containers, preferably amber-colored glass, at a temperature of less than 40 °C (preferably between 15 to 30 ° C). Freezing should be avoided (McEvoy , 2002; Bishop, 2005). The shelf-life is 2 years from the date of manufacturing if kept under the above conditions (as determined by Syphco Co., Syria).

The label of the present preparation of atropine sulphate 0.1% should state the it is a veterinary preparation that can be given s.c., i.m. or i.v. at the dose rates of 0.02-0.045 mg/kg body weight. It should be protect from light and kept between 15-30 °C. The expiry date of the product is usually 24 months from the date of manufacture.

## Conclusion

Atropine sulphate injection solutions which are used in veterinary practice in Iraq and neighboring countries are usually imported. The present findings would be a contribution for the manufacture of 0.1% atropine solution on a commercial basis when needed, as we introduce the know-how of the product intended for veterinary use only.

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## Conflict of interest

Authors declare that they have no conflict of interest.

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