

Side effects of the cough drug dextromethorphan, studied on ants as models

Abstract

Dextromethorphan, the currently preferred cough drug, tested on ants used as biological models, decreased the food consumption of these insects, increased their sinuosity of movement, reduced their tactile (pain) perception, and impacted their social relationships. It did not affect the ants' orientation ability, audacity, cognition, conditioning acquisition and memory. The ants did not adapt themselves to the side effects of dextromethorphan and became dependent on its consumption. The effect of the drug quickly and linearly decreased after weaning, becoming very weak after 4–6 hours and null after 10–12 hours. Dextromethorphan led to dependence, what can also occur in humans. Being safer than previously used cough drugs, dextromethorphan can be consumed for treating dry cough, but in order to prevent dependence, should be used only at therapeutic doses and during a limited time.

Keywords: dependence, food consumption, *Myrmica sabuleti*, sinuosity of movement, social relationships

Volume 6 Issue 1 - 2021

Marie Claire Cammaerts,¹ Roger Cammaerts²

¹Independent researcher, retired from the Biology of Organisms Department, University of Brussels, Belgium

²Independent researcher, retired from the Natural and Agricultural Environmental Studies Department (DEMNA) of the Walloon Region, Belgium

Correspondence: Marie-Claire Cammaerts, Independent researcher, 27, Square du Castel Fleuri, 1170 Bruxelles, Belgium, Tel 32 2 673 49 69, Email mccammaerts@gmail.com

Received: January 25, 2021 | **Published:** February 23, 2021

Abbreviations: ang.deg. = angular degrees; ang.deg./cm = angular degrees per cm; mm/s = millimeter per second; χ^2 = chi-square; vs = versus; n° = number; cm = centimeter; mm = millimeter; ml = milliliter; mg = milligram; g = gram; s = second; min = minute; h = hour; t = time; % = percentage

Introduction

Acute cough impacting the quality of life is often treated by over-the-counter remedies, as there is no specific therapy. Assessing their efficacy can be done using objective effects on clinical outcomes, such as cough counting and cough challenge provoked by protussive agents compared to placebo, as well as by the subjective perception of life improvement.¹ Most cough drugs act as antihistaminics, the side effects of which are those of anticholinergic drugs. One example is promethazine. Being a first-generation antihistaminic, it easily penetrates the blood-brain barrier, causing adverse effects such as central nervous system (CNS) and respiratory depression² as well as dry mouth, constipation, blurred vision, sedation, urinary retention and dermatitis.^{3,4} Moreover, cases of misuse and abuse have been reported for promethazine,⁵ a fact also mentioned in a study on drugs abuse and dependence.⁶

Another cough drug, codeine, belonging to the opioids, has been used since a long time and is still used as a central cough suppressant. Codeine acts as a prodrug, being converted in morphine in the liver by the cytochrome P450 2D6 enzyme. This cytochrome system is however highly polymorphic, what results in uncertainties about the opiate and side effects of the drug. Codeine was found to have no more effect than placebo syrup for cough due to upper respiratory infections (under an objective assessment⁷ as well as under objective and subjective assessments).⁸ It had a positive effect when cough was due to lower respiratory system diseases (under an objective assessment;^{9–11} as well as under objective and subjective assessments),¹² with the exception of a cough challenge trial using citric acid on patients suffering from chronic obstructive pulmonary disease, where it was found to have no more effect than placebo.¹³ In healthy volunteers submitted to a

challenge trial, codeine either significantly suppressed capsaicin-induced cough¹⁴ or failed to suppress it.¹⁵ On the whole, there is little clinical evidence of antitussive activity for codeine.^{1,16}

Being the dextrogyre enantiomer of levomethorphan, which is an opioid analgesic, dextromethorphan is a morphine derivative, acting as a central cough suppressant.¹⁶ It was introduced in the pharmacopeia as a treatment of cough without the side effects of codeine, such as constipation, nausea, drowsiness and dependency, and has been widely used since more than half a century ago. As codeine, it is metabolized by the cytochrome P450 system.¹⁶ Five distinct cough challenge studies with healthy adult volunteers showed that dextromethorphan attenuated cough released by citric acid aerosols, while another challenge study showed no reduction.¹⁶ Besides this, a cough challenge using capsaicin showed a reduction of the cough reflex.¹⁷ A comparative assessment of the efficacy of dextromethorphan and codeine *versus* placebo showed that both drugs caused a significant reduction in cough intensity when the latter was due to lower respiratory diseases (LRD), dextromethorphan scoring better than codeine.¹² Measured by the reduction of cough counts on a small sample of patients, dextromethorphan was also found as efficient as codeine in chronic bronchitis, when compared with placebo.¹¹ On the contrary, another study on patients with LRD showed no reduction in cough counts.¹⁸ These three studies on LRD were performed on small samples of patients (8 to 28). In upper respiratory infections (URI), dextromethorphan appeared to be efficient compared with placebo, when the efficiency was measured by the reduction of cough counts.^{19,20} However, in two other studies conducted on URI cases, dextromethorphan was not found efficient in reducing cough frequency or severity.^{21,22} Another study, measuring cough frequency and sound pressure as well as subjective scores for cough severity on patients suffering from URI provided little significant difference between dextromethorphan and placebo.²³ To explain these discrepancies, Pavesi et al.²⁰ suggested that in order to demonstrate a statistically significant effect, the different dextromethorphan efficacy assessments should require larger numbers of subjects.

Nevertheless, on the basis of these previous results, Bolser²⁴ recommends cough suppressants for the short-term symptomatic relief of coughing in patients suffering from chronic bronchitis while he does not recommend their use for URI as they have only limited efficacy for symptomatic relief. A subjective parental assessment of children's upper respiratory cough and sleep as well as of their parents' sleep quality showed no significant difference between placebo and dextromethorphan or diphenhydramine treatments and even that insomnia was more frequent under dextromethorphan treatment.²⁵ The authors of this work conclude that practitioners should consider their observations as well as the cost of the drug and its potential adverse effects before treating humans with dextromethorphan. Another subjective assessment of nocturnal cough and sleep difficulty in 6 to 18 years old children with upper respiratory infections concluded to a similar lack of efficacy of dextromethorphan, diphenhydramine and placebo.²⁶ A comparison of the efficacy of dextromethorphan, of a dextromethorphan-salbutamol combination and placebo on cough frequency and severity showed that the combination treatment was more efficient in suppressing cough at night, but that no difference between the three treatments appeared as for the reduction of cough during the day. The authors conclude that the use of antitussives is usually unnecessary.²¹

Interestingly, an assessment of children cough frequency and severity as well as of children and parents subjective opinions on sleep quality showed that honey consumed before sleeping appeared to be as efficient as dextromethorphan for calming nocturnal cough.^{27,28}

With dextromethorphan taken in the usual moderate therapeutic doses (20 - 30 mg for human adults, 0.2 – 0.4 mg for children per day), common adverse effects are reported in Rühle et al.¹⁸, in the Medscape data base²⁹ and in the instructions for use joined to the drug packages. They are nausea, vomiting, diarrhea, blurred vision, constipation, drowsiness, dizziness, as well as sedation, confusion and nervousness, thus essentially gastrointestinal and CNS impairments. Moreover, as a consequence of its easy availability and mode of action on CNS, cases of abuse and overdose consumption are reported, leading to more significant adverse events, such as the development of dependence on its consumption (chronic addiction),^{30,31} psychosis including delusions, hallucinations and paranoia,³² stress and decrease of motor coordination and of the notion of fear.³³ In addition, due to the polymorphism of the major dextromethorphan metabolic catalytic pathway, the P450 2D6 enzyme, ca 5% of the Europeans lack the ability to metabolize dextromethorphan and are thus able to suffer from acute intoxication.³⁴

No information could be found in the literature as for the effect of dextromethorphan on the individuals' cognition, learning, memory, social relationships, and adaptation to side effects. We here intend to fill these gaps by examining the potential side effects of dextromethorphan on several physiological and ethological traits of ants used as biological models as has been done for other substances used by humans.³⁵⁻³⁹ Here below we explain why ants can be used as models, which species was used, what we know on it, and which biological traits we intend to examine. Ants can be used as biological models for the following reasons. Most biological processes are similar in every animal including human. Several vertebrates and invertebrates are thus used as models.^{40,41} Invertebrates are advantageously used since they are small, are easily maintained in a laboratory; have a short life cycle and have a rather simple anatomy.⁴² Insects are among the most used invertebrates, and among them, the social hymenoptera.⁴³ The ants are particularly appropriated to be used since they present many sophisticated biological traits, e.g.

they navigate using learned cues, recruit nestmates, differently mark parts of their territory, take care of their brood, build complex nests, clean them and manage cemeteries at the frontiers of their territory.⁴⁴ The biology of the ant *Myrmica sabuleti* Meinert, 1861 is rather well known, particularly its recruitment strategy, visual perception, navigation system, visual and olfactory conditioning ability,⁴⁵ and the ontogenesis of several of their cognitive abilities.⁴⁶ Workers of this ant recognize themselves in a mirror, become imprinted at their emergence to the appearance of the front head of their congeners, learn their alarm reaction and trail following behavior during their first year of life in the presence of older congeners,^{46,47} natively possess a number line, acquire the notion of zero through experiences, and can acquire and use numerical symbolisms.^{48,49} Distance and size effects as well as Weber's law can be applied to their perception and reactions.^{50,51} They can anticipate when and where the next food distribution will take place,^{52,53} as well as whether the next quantity of an increasing or decreasing numerical sequence presented over time will be larger or smaller.⁵⁴ Their cognitive abilities however always stay at a concrete level and never reach an abstract one.

Here, the ant *M. sabuleti* was again used as a biological model for examining the effect of dextromethorphan on the workers' food consumption, general activity, locomotion, orientation ability, audacity, tactile (pain) perception, social relationships, cognition, stress, learning ability and memory, as well as the adaptation to the side effects of the drug, the dependence on its consumption, and the decrease of the effect of the drug after its consumption was stopped. The experimental protocols were identical to those previously used e.g. 55-57. Therefore, we here only briefly explain them, being however unable to avoid inevitable plagiarism. We want to state that we took knowledge of the literature relating information on the side effects of dextromethorphan only after having finalized our experimental work.

Material and methods

Collection and maintenance of ants

The experiments were made on three colonies of *M. sabuleti* collected in autumn 2019 in an abandoned quarry located at Olloy/Viroin (Ardenne, Belgium). The colonies were living under stones and in grass and contained ca 500 workers, a queen and brood. Each colony was maintained in the laboratory in one to three glass tubes half filled with water, with a cotton plug separating the ants from the water. These nest tubes were deposited in a tray (34 cm x 23 cm x 4 cm) which constituted the ants' foraging area. In these trays, pieces of *Tenebrio molitor* larvae (Linnaeus, 1758) were deposited three times per week, and a 30% aqueous solution of sugar was permanently provided in a tube plugged with cotton. The lighting of the laboratory equaled ca 330 lux, the ambient temperature ca 20°C, the humidity ca 80%, and the electromagnetism 2 μWm^2 . These environmental conditions were suitable for the species. The ants are commonly referred as 'workers' or 'nestmates' as usually done for social insects.

Solution of dextromethorphan given to the ants

Pure powder of dextromethorphan hydrobromide (25 g) produced by FSA Chemicals (2960 Belgium, www.fsa-chemicals.be), was provided by the pharmacist Wera (Brussels, Belgium). Humans who need to use this drug are advised to daily consume 20 mg of it. At the same time, humans absorb about one liter of water per day. Insects, and so ants, consume about ten less water than mammals, due to their specific physiology and anatomy. Therefore, to set ants under a dextromethorphan diet equivalent to that of humans, they were

provided with a solution of 20 mg dextromethorphan in 100 ml of the sugar water permanently at their disposal in cotton plugged tubes. The cotton plug of these tubes was refreshed every 2-3 days, and their content was renewed every 7 days. We checked several times per day if ants of each colony drunk the furnished solutions, and effectively they did. To conduct the present work, firstly, all the control experiments except the one concerning the ants' conditioning ability and memory were made on two colonies labeled A and B maintained under normal diet. The control experiments relative to conditioning and memory as well as to brood caring were made on colony C, also maintained under normal diet. Then, the colonies were provided with the drug solution, and the test experiments were conducted on colonies A and B, except the experiment relative to brood caring, which was conducted on colony C. The experiments started after the ants had consumed the drug during one day, and ended after 12 days when the loss of the effect of dextromethorphan after weaning had been studied.

Meat and sugar water consumption, general activity

While the ants were under normal diet, then under a diet containing dextromethorphan, we counted six times per day during six days (i.e. four times during the day and two times during the night, each day at the same times o'clock), the ants of colonies A and B present on their meat, at the entrance of their sugar water tube, and those moving in their foraging area, at the entrance of their nest and inside of it. For each two kinds of diet and each three kinds of trait (eating meat, drinking, being active), the mean of the 6 times x 2 colonies = 12 counts was established (Table 1, the six first lines). To statistically analyze the recorded data, for each three kinds of trait, the six daily means obtained for ants under dextromethorphan diet were compared to the six corresponding daily means obtained for ants under normal diet using the non-parametric test of Wilcoxon, the level of significance being set at $P = 0.05$.⁵⁸ The means of these six daily means were also calculated (Table 1, last line).

Table 1 Impact of dextromethorphan on the ants' food consumption and general activity

Days	Normal Diet			Diet with dextromethorphan		
	Meat	Sugar water	Activity	Meat	Sugar water	Activity
I	1.50	1.00	7.5	1.25	1.00	11
II	1.50	3.00	8.5	1.00	1.00	6.5
III	1.50	1.75	9.5	1.50	0.5	7.25
IV	1.75	2.25	9.5	1.25	1.00	8.50
V	1.75	2.50	10.5	1.00	1.00	9.75
VI	2.00	2.00	9.92	1.25	0.50	7.00
mean	1.67	2.08	9.24	1.21	0.83	8.33

The table gives, for each day, the mean of 12 counts of ants eating meat, drinking sugar water, and being active (lines I to VI), as well as the mean of these six means (last line). Dextromethorphan decreased the ants' food consumption and activity. Details and statistics are given in the text

Linear and angular speeds, orientation

These three traits were quantified on ants walking in their foraging area. The speeds were assessed without stimulating the ants, and the orientation when stimulating the ants with a nestmate tied to a piece of paper (Figure 2A). Such a tied nestmate emits its attractive mandible glands alarm pheromone. The ants' speeds were recorded during one experiment and their orientation during another one. Each time, the trajectories of 40 foragers were recorded and analyzed using appropriate software⁵⁹ established on the basis of the following definitions. The linear speed (in mm/s) is the length of a trajectory divided by the time spent to travel it; the angular speed (in ang.deg/cm) is the sum of the angles made by successive adjacent segments, divided by the length of the trajectory; the orientation (in ang.deg.) towards a location is the sum of successive angles made by the direction to the location and the direction of the trajectory, divided by the number of measured angles. When an animal tends to orient itself towards the location, the obtained value of orientation is lower than 90° . When an animal tends to avoid the location, the obtained value is larger than 90° . The median and quartiles of the distribution of the 40 obtained values were established for the linear speed, the angular speed, and the orientation (Tables 2, lines 1, 2, 3). The distributions obtained for ants under dextromethorphan diet were compared to the corresponding distributions obtained for ants under normal diet using the non-parametric χ^2 test.⁵⁸

Audacity

A squared platform (9 cm²) bearing a cylindrical tower (height = 4 cm; diameter = 1.5 cm), both in white Steinbach® paper, was deposited in the ants' foraging area. The ants present on this unknown

risky apparatus were counted 10 times over 10 minutes (Figure 2 B). The mean and extremes of the recorded numbers were established (Table 2, line 4). To analyze the results, the numbers of ants of the two colonies counted during every two successive minutes were added, and the five sums obtained for ants under dextromethorphan diet were compared to the five ones obtained for ants under normal diet using the non-parametric test of Wilcoxon.⁵⁸

Tactile (pain) perception

Under normal diet, the ants perceiving the uncomfortable character of a rough substrate walk on it with difficulty, slowly, sinuously, and often touch it with their antennae (Figures 2 C1). Ants poorly perceiving the uncomfortable character of such a substrate walk on it more easily, more quickly and less sinuously. Therefore, to assess the ants' tactile perception, a folded piece (3 cm x 2 + 7 + 2 = 11 cm) of n° 280 emery paper was tied to the bottom and the borders of a tray (15 cm x 7 cm x 4.5 cm) which became divided into 3 zones, a first 3 cm long one, a second 3 cm long one covered with the emery paper, and a last 9 cm long one without emery paper. To make an experiment on one colony, 12 ants were deposited in the first zone of the apparatus, and their trajectories on the emery paper were recorded. Working on colonies A and B, a total of 24 trajectories were recorded, and the ants' linear and angular speeds assessed as usually (see subsection relative to linear and angular speeds). The median and quartiles of the distributions of the 24 obtained values were established for each variable and each kind of diet (Table 2, lines 5, 6). The distributions obtained for ants consuming dextromethorphan were compared to those obtained for ants under normal diet using the non-parametric χ^2 test.⁵⁸

Brood caring

Because retrieving larvae caused a trauma to the colony, this trait was examined only on colony C, and twice in order to observe $2 \times 5 = 10$ larvae at a time. Each time, a few larvae were removed from the nest and deposited in front of the entrance. Five of them were observed during five minutes (Figure 2 D), and those among these 5 larvae not re-entered after 30 seconds, 1, 2, 3, 4 and 5 minutes were counted. The numbers obtained during the two experiments were added, the total number of observed larvae being 10 (Table 3, line 1). The six sums obtained for ants consuming dextromethorphan were compared to the six sums obtained for ants maintained under normal diet using the non-parametric test of Wilcoxon.⁵⁸

Social relationships

Nestmates normally present no aggressiveness towards one another, but drugs may affect this peaceful social relationship. For examining the impact of dextromethorphan on the ants' social relationship, for ants under normal diet then for those consuming the drug, five dyadic encounters were performed on ants of colonies A and B (10 encountering in total). Each encounter occurred in a cup (diameter = 2 cm, height = 1.6 cm), the borders of which having been slightly covered with talc. During each encounter, one ant of the pair was observed during 5 min and its behavior was characterized by the numbers of times it did nothing (level 0 of aggressiveness), touched the other ant with its antennae (level 1), opened its mandibles (level 2), gripped and/or pulled the other ant (level 3), tried to sting or stung the other ant (level 4) (Figure 2 E). The numbers of cases of each behavior obtained for the two colonies were added (Table 3, line 2), and the sums obtained for ants under dextromethorphan diet were compared to those obtained for ants under normal diet by using the non-parametric χ^2 test.⁵⁸ The ants' behavior was also characterized by a variable 'a', which equaled the number of aggressiveness levels $2 + 3 + 4$ divided by the number of levels $0 + 1$.

Cognition

This trait was assessed by setting 15 ants of colony A and of colony B in an own tray (15 cm x 7 cm x 4.5 cm) into which two duly folded pieces of extra strong white paper (Steinbach®, 12 cm x 4.5 cm) had been inserted managing so a twist and turns path between a small 2 cm long zone in front of that path and a 8 cm long zone beyond the path. A small bit of cotton wool dampened with water was present in the latter zone. The 15 ants were set all together in the small area. After that, the ants still there and those having reached the zone beyond the twists and turns path were counted after 2, 4, 6, 8, 10 and 12 minutes (Figure 2 G). The numbers obtained for the two colonies were added (Table 3, line 3). For ants counted in each of the two areas, the six sums obtained for ants consuming dextromethorphan were statistically compared to those obtained for ants under normal diet using the non-parametric Wilcoxon test.⁵⁸

Escaping behavior

For colony A and colony B, six of their ants were enclosed under a reversed polyacetate cup (h = 8 cm, bottom diameter = 7 cm, ceiling diameter = 5 cm) deposited in the foraging area. The inner surface of the glass had been slightly covered with talc to prevent the ants climbing. The ants were introduced into this enclosure through a hole (diameter = 3 mm) made in its ceiling, and a notch (3 mm height, 2 mm broad) had been made in the rim of the bottom of the cup to allow the ants escaping (Figure 2 F). The ants which could escape were counted after 2, 4, 6, 8, 10 and 12 minutes, and the numbers obtained for the two colonies were added (Table 3, line 4). The six

sums obtained for ants consuming dextromethorphan were compared to those obtained for ants living under normal diet by using the non-parametric Wilcoxon test.⁵⁸

Conditioning acquisition, memory

For colony C maintained under normal diet, and later on for colonies A and B maintained under dextromethorphan diet, at a given time, a yellow hollow cube build in strong paper (Canson®) was placed above the entrance of the sugar water tube. Since that time, the ants underwent operant visual conditioning. Colony C was used to obtain the control result because once individuals become conditioned to a cue, they are no longer naive for that cue, and their conditioning to that cue can no longer be assessed. From the cube deposit, the ants were tested in the course of their conditioning acquisition, then, from the cue removal, in the course of the loss of their conditioning. The tests were performed in a Y-apparatus made of strong white paper, set in a tray, the sides of the Y-apparatus having been slightly covered with talc to prevent escaping. The floor of the Y-apparatus was covered with a thin paper replaced between each test. Each Y-apparatus was provided with a yellow hollow cube in one of its branches, half of the tests being made with the cube in the left branch and the other half with the cube in the right branch of the Y-maze.

To make a test, 10 ants of colony C, or 10 ants of colony A and 10 ants of colony B, were individually tested in the Y-maze. Each ant was deposited in the area lying in front of the two branches of the apparatus, and the ants' first choice of one or the other branch of the Y-apparatus was recorded (Figure 2 H). Choosing the branch containing the yellow cube was considered as giving the correct response. After having been tested, the ant was kept in a cup until 10 ants of its colony were tested, for not testing twice the same ant. After the test, the 10 ants were replaced into their foraging area, near their nest entrance. For each test, the choices of ants of colonies A and B were added ($n = 10$ ants x 2 colonies = 20 responses). The numbers of correct responses obtained for these ants consuming dextromethorphan during their acquisition then their loss of conditioning were statistically compared to the numbers obtained for the ants of colony C living under normal diet, by using the non-parametric Wilcoxon test.⁵⁸ The proportions of correct responses all along the experiment were also calculated (Table 4).

Adaptation to side effects of dextromethorphan

An individual becomes adapted to a drug or a situation when he less and less suffers over time from the side effects of the drug or situation. For studying such an event, a trait impacted by the drug or the situation must be assessed after a short then a long time of use, and the results of these two assessments compared. Dextromethorphan largely impacted the ants' angular speed. This trait was thus assessed after 7 days of drug consumption as it had been assessed after one day of consumption (Table 5, line 1), and the result of the assessment made after 7 days was compared to the control one and to that made after one day using the non-parametric χ^2 test.⁵⁸

Habituation to wanted effects of dextromethorphan

An individual becomes habituated to a drug or a situation when he becomes less and less sensitive to the wanted effect of this drug or situation. To study such an event, a trait improved by the drug or the situation should be assessed after a short then again after a long time of use and the results of the two assessments compared. In the present study, none of the ants' examined traits was found to be improved by dextromethorphan consumption. Habituation to this drug could thus not be examined.

Dependence on dextromethorphan consumption

An individual becomes dependent on a drug or a situation when he wants continuing using this drug or situation and even can no longer live without them. In the present work, dependence on dextromethorphan was examined after the ants had consumed it during 9 days. For colony A and for colony B, 15 ants were deposited in a tray (15 cm × 7 cm × 5 cm) containing two cotton-plugged tubes (h = 2.5 cm, diam. = 0.5 cm), one filled with sugar water, the other with the sugared solution of dextromethorphan used over the experimental

work. The tube containing the drug was located on the right in the tray of one colony, and on the left for the other colony (Figure 2 I). In the course of 15 minutes, the ants of each colony approaching each tube were counted 15 times, and the 15 corresponding counts obtained for the two colonies were added (Table 5, line 2). These sums allowed calculating the proportion of ants having chosen the drug-free solution and that containing the drug. The two sums of counts (= two numbers) were compared to the two numbers expected if the ants randomly visited the two presented tubes, using the non-parametric χ^2 goodness-of-fit test.⁵⁸

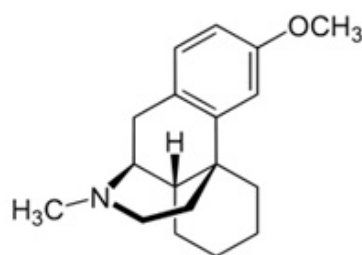


Figure 1 Upper part: chemical structure of dextromethorphan, the side effects of which were examined in the present work using ants as models. Lower part: material used to make the solution of the drug given to the ants, and two ants drinking this solution.

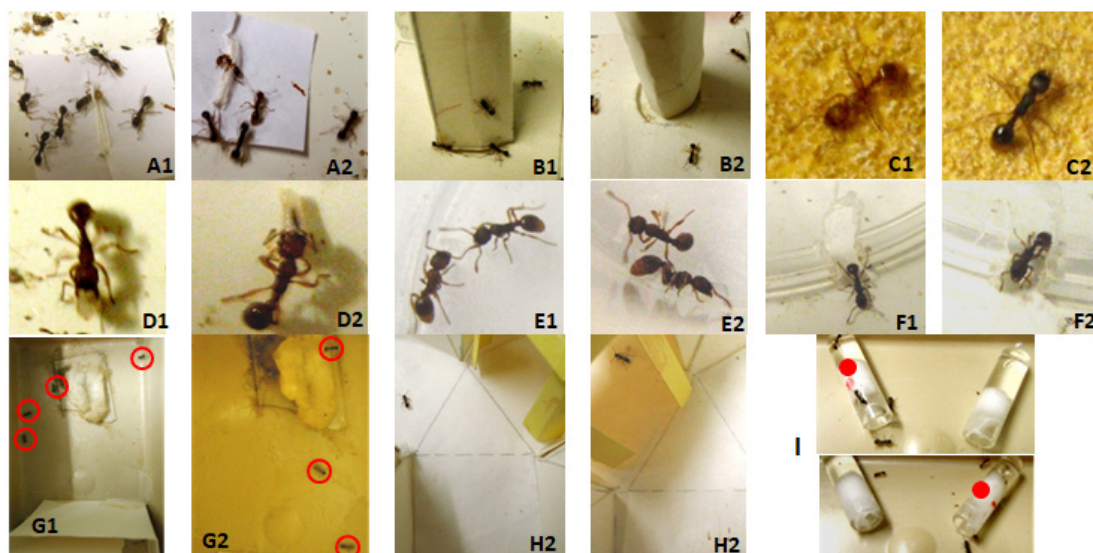


Figure 2 Some views of the experiments. 1: ants under normal diet; 2: ants under a diet with dextromethorphan. A: ants having come near a tied nestmate which emitted its attractive alarm pheromone. B: ants coming onto an unknown risky apparatus. C: ants walking on a rough substrate, with difficulty and touching the substrate with the antennae while being under normal diet, and more frankly and not touching the substrate with the antennae while under drug influence. D: an ant under normal diet holding a larva in its mandibles, and an ant under dextromethorphan diet in front of a larva and not holding it. E: two nestmates under normal diet staying near one another, and two ones consuming dextromethorphan avoiding themselves. F: an ant under normal diet and one under a diet with dextromethorphan going out of an enclosure. G: ants living under normal diet as well as ants consuming dextromethorphan having been able to cross a path with twists and turns. H: two ants under dextromethorphan diet, trained to a yellow cube, and giving, one the wrong response, the other the correct response when tested in a Y-apparatus provided with a yellow cube. I: ants of colonies A and B maintained under dextromethorphan and preferring a solution containing the drug (red dot) than a drug-free solution.

Table 2 Impact of dextromethorphan on five ants' physiological and ethological traits

Traits	Normal diet	Diet with dextromethorphan
Linear speed (mm/s)	11.3 (10.1 – 12.6)	10.4 (9.9 – 11.9)
Angular speed (ang.deg./cm)	109 (96 – 127)	164 (145 – 184)
Orientation (ang.deg.)	28.3 (23.8 – 37.4)	31.5 (24.7 – 40.5)
Audacity (n°)	2.80 [2 – 4]	2.25 [1 – 4]
Tactile perception		
Linear speed (mm/s) on a rough substrate	5.1 (4.5 – 5.9)	6.7 (6.2 – 7.7)
Angular speed (ang. deg./cm) on a rough substrate	245 (223 – 272)	184 (169 – 197)

The table gives the median (and quartiles) or the mean [and extremes] of the recorded values. Dextromethorphan increased the ants' sinuosity of movement and decreased their tactile perception. Details and statistics are given in the text. mm = millimeter, s = second, ang.deg. = angular degrees, cm = centimeter, n° = number

Table 3 Impact of dextromethorphan on four physiological and ethological traits

Traits	Normal diet	Diet with dextromethorphan
Brood caring: n° of not re-entered larvae over time	½ 1 2 3 4 5 min 9 8 6 4 1 0	½ 1 2 3 4 5 min 10 9 8 6 4 2
Social relationships: n° of levels of aggressiveness; variable 'a'	levels 0 1 2 3 4 'a' 71 64 19 0 0 0.14	levels 0 1 2 3 4 'a' 25 41 52 0 0 0.79
Cognition: n° of ants in front and beyond a twists and turns path over time	2 4 6 8 10 12 min in front 21 19 16 13 10 9 beyond 0 0 1 4 6 7	2 4 6 8 10 12 min in front 21 19 17 14 12 13 beyond 0 1 2 4 5 6
Escaping ability: n° of escaped ants over time	2 4 6 8 10 12 minutes 2 4 5 7 10 12	2 4 6 8 10 12 minutes 2 5 6 7 9 11

Dextromethorphan impacted the ants' brood caring and social relationships, but not their cognition and escaping ability. Details and statistics can be found in the text. n° = number, min = minute

Table 4 Impact of dextromethorphan on ants' conditioning ability and memory

Time	Normal diet, colony C. %	Diet with dextromethorphan, colony A ; colony B. %
7 hours	50%	6 vs 4 ; 6 vs 4 60%
24 hours	65%	7 vs 3 ; 6 vs 4 65%
31 hours	70%	7 vs 3 ; 7 vs 3 70%
48 hours	70%	6 vs 4 ; 8 vs 2 70%
55 hours	75%	7 vs 3 ; 8 vs 2 75%
72 hours	80%	8 vs 2 ; 7 vs 3 75%
Cue removal		
7 hours	70%	9 vs 1 ; 7 vs 3 80%
24 hours	70%	8 vs 2 ; 6 vs 4 70%
31 hours	70%	7 vs 3 ; 7 vs 3 70%
48 hours	70%	7 vs 3 ; 7 vs 3 70%
55 hours	70%	7 vs 3 ; 7 vs 3 70%
72 hours	70%	7 vs 3 ; 7 vs 3 70%

Dextromethorphan did not affect the ants' conditioning ability, and thus their short-term memory, and did not impact their middle and long-term memory since they remembered the learned cue for more than 72 hours. % = proportion; vs = versus (number of correct responses versus number of wrong responses)

Table 5 Ants' adaptation to the effect of dextromethorphan on their locomotion; ants' dependence on this drug consumption

Adaptation to the impact of the drug on locomotion	Normal diet	Under the drug diet since 2 days	Under the drug diet since 7 days
Linear speed	11.3 (10.1 – 12.6)	10.4 (9.9 – 11.9)	9.3 (8.8 – 10.8)
Angular speed	109 (96 – 127)	164 (145 – 184)	178 (168 – 197)
Dependence on dextromethorphan consumption	Colony A	Colony B	Total
Drug-free solution	11	5	16 (20.25%)
Solution with the drug	28	35	63 (79.75%)

The ants did not adapt themselves to the impact of the drug on their locomotion: their linear speed and their sinuosity of movement stayed impacted by the drug. The ants became dependent on the drug consumption: they preferred a liquid containing it than a drug-free liquid. mm/s = millimeter per second, ang.deg./cm = angular degrees per centimeter, % = proportion

Decrease of the effect of dextromethorphan after weaning

This decrease was studied after the ants had consumed the drug for 12 days, and the study was based on the effect of the drug on the ants' angular speed, a trait largely impacted by the drug and easily quantified in a short time. The ants received a fresh solution of dextromethorphan 12 hours before weaning. At this time, at $t = 0$ h, the ants' angular speed was assessed as it had been after 1 and 7 days of consumption except that 20 instead of 40 trajectories were recorded and analyzed. After this assessment, weaning started: the sugared solution containing the drug was replaced by a drug-free

sugared solution. From this time onwards, the ants' angular speed was assessed every two hours until it became statistically similar to that of ants under normal diet (Table 6, Figure 3). The distributions of the angular speed values obtained over time after weaning were compared to that obtained at $t = 0$ as well as to that obtained for ants under normal diet by using the non-parametric χ^2 test for independent samples⁵⁸ The resulting P values were adjusted for multiple comparisons by using the Benjamini-Hochberg procedure⁶⁰ with a false discovery rate of 0.05. For calculation of the adjusted P values, < 0.001 was considered as = 0.001 and for the other P values interpolation between critical values was used. The function describing the regression of the ants' angular speed over time was established using Statistica® v.10 software.

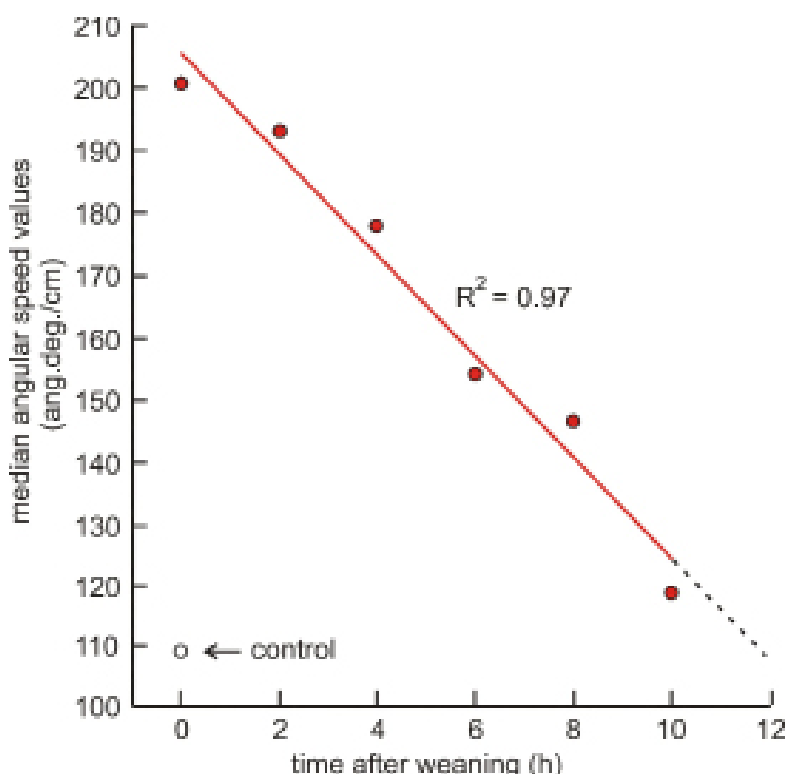


Figure 3 Decrease of the effect of dextromethorphan after weaning. The effect of the drug rapidly and linearly decreased in a total of about 10 hours. It significantly differed from the initial value 6 hours after weaning, and no longer significantly from the control one 10 hours after weaning. The extended regression line precisely reached the control value 12 hours after weaning. The rapid and steep decrease accounted for the development of dependence on dextromethorphan consumption. Numerical and statistical results are given in Table 6, and details can be found in the text. ang.deg./cm = angular degrees per centimeter.

Table 6 Decrease of the effect of dextromethorphan after weaning

Time (h)	Angular speed	versus t = 0			statistics			versus control		
	ang.deg./cm	χ^2	df	P		χ^2	df	P		
t = 0	201 (178 – 217)									
2h	193 (174 – 213)	0.85	2	P = 0.665		47.96	1	P = 0.003		
4h	178 (171 – 196)	3.14	2	P = 0.275		35.74	1	P = 0.003		
6h	154 (149 – 180)	26.75	2	P = 0.002		9.41	1	P = 0.003		
8h	147 (129 – 160)	18.45	1	P = 0.002		5.33	1	P = 0.028		
10h	119 (102 – 134)	26.67	1	P = 0.002		2.02	2	P = 0.386		
control	109 (96 – 127)									

The effect of the drug rapidly decreased in a total of 10 hours. Six hours after weaning, the effect significantly differed from the initial one, and ten hours after weaning, it was not significantly different from the control one. Details are given in the text, and an illustration is shown in Figure 3. t = time, h = hours, ang.deg./cm = angular degrees per centimeter. Benjamini-Hochberg adjusted P values

Results

Meat and sugar water consumption, general activity

These physiological traits were impacted by dextromethorphan consumption (Table 1). While consuming this drug, the ants eat less meat and drunk less sugar water than when living under normal diet, these two observations being significant (each time: $N = 5$, $T = -15$, $P = 0.031$). Moreover, ants consuming dextromethorphan were less active than when not consuming this drug, but this difference was not significant ($N = 6$, $T = -15$, $P = 0.219$) because this impact on activity occurred with some delay, being not yet efficient during the first assessment of the activity.

Linear and angular speeds

The ants' linear speed slightly decreased under dextromethorphan consumption, but not significantly (Table 2, line 1; $\chi^2 = 2.62$, $df = 2$, $0.20 < P < 0.30$). The ants' angular speed (= sinuosity) increased significantly under dextromethorphan consumption (Table 2, line 2; $\chi^2 = 49.42$, $df = 2$, $P < 0.001$), the increase being obvious to observer. This large increase of sinuosity can explain the simultaneous slight decrease of linear speed.

Orientation

The ants' orientation ability was slightly, although not significantly, impacted by dextromethorphan consumption (Table 2, line 3; $\chi^2 = 0.62$, $df = 1$, $0.30 < P < 0.50$; Figure 2 A1 A2). This slight decrease may be explained by the ants' larger sinuosity of movement under dextromethorphan diet.

Audacity

This trait was not significantly impacted by dextromethorphan consumption (Table 2, line 4; $N = 5$, $T = -13.5$, $P = 0.78$). Under that diet, the ants still came onto the presented unknown apparatus but soon went away from it (Figure 2 B1 B2).

Tactile (pain) perception

This physiological trait was impacted by dextromethorphan consumption. On a rough substrate, the ants consuming this drug walked more frankly than those living under normal diet, the latter walking with difficulty, slowly, sinuously, and often touching the substrate with their antennae (Figure 2 C1 C2). This was obvious to the observer, and confirmed by the numerical results (Table 2, lines 5, 6) and the statistical analysis (linear speed: $\chi^2 = 16.45$, $df = 1$, $P < 0.001$; angular speed: $\chi^2 = 25.12$, $df = 2$, $P < 0.001$).

Brood caring

Dextromethorphan impacted this behavioral trait (Table 3, line 1; Figure 2 D1 D2). While ants under normal diet rapidly re-entered the larvae experimentally removed from the nest, those consuming the drug did it with some delay. The difference between one and the other kinds of diet as for the kinetic of larvae re-entering was significant ($N = 6$, $T = 21$, $P = 0.016$). This may be due to the lower activity of the ants consuming dextromethorphan, and/or their lower perception, and/or some impact of the drug on the ants' social relationship. The two first explanations have been previously pointed out (see the above subsections relative to activity and tactile perception). The third explanation was examined thanks to the next experiment.

Social relationships

This ethological trait was affected by dextromethorphan consumption (Table 3, line 2). While ants living under normal diet often stayed aside, contacting each other with their antennae, those consuming the drug avoided each other and very often opened their mandibles (Figure 2 E1 E2). Even if not strongly aggressive, the ants consuming dextromethorphan behaved towards one another statistically differently than ants not consuming this drug ($\chi^2 = 38.27$, $df = 2$, $P < 0.001$). This impact of the drug on the ants' social relationship confirmed thus the presumption related in the experiment on the ants' brood caring.

Cognition

This trait was not impacted by dextromethorphan consumption (Table 3, line 3; Figure 2 G1 G2). In front of a twists and turns path, the ants living under normal diet as well as those consuming the drug engaged in the difficult path. The difference between the numbers of ants under one and the other kinds of diet and still in front of the difficult path was at the limit of significance ($N = 4$, $T = 10$, $P = 0.063$), very probably due to the lower activity of the ants consuming dextromethorphan. There was no statistical difference ($N = 4$, $T = 5$, $P = 0.563$) as for the number of ants having reached the zone located beyond the twists and turns path, what clearly showed that the drug did not impact the ants' cognition. This last deduction was checked thanks to the two following experiments.

Escaping behavior

Dextromethorphan did not affect this ethological trait (Table 3, last line). Ants living under that diet could escape from the enclosure just like those living under normal diet (Figure 2 F1 F2) ($N = 4$, $T = 5$, P

= 0.563). This confirmed the result of the previous experiment (see here above), i.e. dextromethorphan did not impact the ants' cognition.

Conditioning ability, memory

Dextromethorphan did not impact the ants' conditioning ability (Table 3, the six first lines). Ants consuming this drug acquired conditioning like those living under normal diet, with no statistical difference ($N = 2$, NS). Also, dextromethorphan did not affect the ants' memory, those living under this drug diet remembering the learned cue as well as ants living under normal diet (Table 4, the last six lines; $N = 1$, NS).

Adaptation to the effect of dextromethorphan on locomotion

Ants did not adapt themselves to the impact of dextromethorphan on their locomotion (Table 5, upper part). After 7 days of that drug consumption, the ants' linear speed was lower than the control one ($\chi^2 = 29.91$, $df = 2$, $P < 0.001$) and even lower than that presented after 2 days of consumption ($\chi^2 = 17.86$, $df = 3$, $P < 0.001$). In the same way, the ants' angular speed presented after 7 days on dextromethorphan diet was larger than the control one ($\chi^2 = 48.22$, $df = 2$, $P < 0.001$) and even larger than that presented after 2 days of consumption ($\chi^2 = 10.13$, $df = 2$, $0.001 < P < 0.01$). This absence of adaptation to the side effect of dextromethorphan is not in favor of its use. It allowed examining the loss of the effect of the drug after weaning on the basis of its impact on the ants' sinuosity (see the below subsection relative to the decrease of effect).

Dependence on dextromethorphan consumption

The ants became dependent on dextromethorphan consumption (Table 5, lower part; Figure 2 I). Having the choice between a solution containing the drug and a drug-free solution, 28 ants of colony A and 35 ants of colony B chose the former solution while 11 ants of colony A and 5 ants of colony B chose the latter solution. In total, 79.75% of the ants' choices were for the solution containing dextromethorphan and 20.25% were for the drug-free solution. The two colonies behaved in the same way as for their choice (χ^2 test for two independent samples = 2.12; $df = 1$, $0.10 < P < 0.20$) and the numbers of ants counted on one and the other kinds of tube statistically differed from the numbers expected if ants randomly visited the two sugar solutions ($\chi^2 = 14.06$, $df = 1$, $P < 0.001$). Such dependence is not in favor of dextromethorphan use.

Decrease of the effect of dextromethorphan after weaning

Numerical and statistical results are given in Table 6 and illustrated in Figure 3. The effect of dextromethorphan rapidly and linearly decreased after weaning what accounted for the development of dependence on its consumption (see the above subsection). The drug stayed active during four hours after weaning, but at 6h its effect was significantly lower ($P = 0.002$) than its initial one. The drug remained then somewhat active since, at 8h, the ants' angular speed still differed from the control one ($P = 0.03$). Ten hours after weaning, the ants' sinuosity was no longer significantly different from the control one. Figure 3 shows that the extended regression line exactly reaches the control value 12 hours after weaning.

This decrease was linear and is described by the function:

ants' sinuosity (ang. deg./cm) = $206 - 8.17 t$, with t = time (hours), with $R^2 = 0.97$.

Discussion – conclusion

Dextromethorphan provided to ants in a concentration corresponding to the usual therapeutic dose administrated to adult humans affected their food consumption, activity, sinuosity of movement, tactile (pain) perception, and social relationships. It did not impact their orientation ability, audacity, cognition, conditioning acquisition and memory. Ants did not adapt themselves to the side effects of dextromethorphan. They became dependent on this drug consumption, the effect of which rapidly decreased after weaning, becoming poorly efficient after 6 hours and totally inefficient in *ca* 10–12 hours. Habituation to a wanted effect of dextromethorphan on ants could not be examined, but it is known in humans⁶¹ (see here below).

In humans, consumption of dextromethorphan can lead to gastrointestinal side effects (nausea, vomiting, constipation) as well as to side effects on CNS (drowsiness, dizziness, confusion and nervousness) and immune reactions (rash, angioedema, bronchospasms).^{29,62} These effects may possibly correspond to the ants' decrease of food consumption, increase of sinuosity of movement and decrease of tactile (pain) perception. No adverse effect is reported on humans' cognition, learning and memory, and a same absence of side effect was observed on the ants' cognition, conditioning acquisition and memory. Also, nothing is reported in humans about social relationships impairment and adaptation to the side effects of dextromethorphan. We showed that the social relationships of the ants were affected by dextromethorphan consumption and that these insects never adapted themselves to the side effects of this drug. Moreover, they became dependent on this drug consumption. In humans, the elimination half-life of the drug is about 4 hours in extensive metabolizers,²⁹ what agrees with the short action time measured in ants and should account for dependence. Indeed, some cases of tolerance to dextromethorphan hydrobromide consumption are known in humans, and this leads to dependence. The consequence is an increased dosing over time and, ultimately bromide intoxication.⁶¹

Pharmacists should have expected the humans' abuse of dextromethorphan in order to have hallucinogenic effects similar to those released by opioid drugs, a process culminating in complete dissociation from one's body at doses > 240 mg, this effect lasting 6 hours.³⁰ Instructions for use⁶³ clearly recommend consuming dextromethorphan at therapeutic dosage, i.e. for adults, not to exceed 68 to 90 mg in 3 to 4 daily intakes spaced at least 4 hours apart for a maximum of 5 days. This enables to avoid problems similar to those resulting from the consumption of opiate drugs. The ants we experimented were maintained under dextromethorphan diet during 12 days, and during this time the experiments were conducted without interruption in order to not increase this time period. During the last experiments, relative to cognition and conditioning acquisition, we may have observed impacts of the drug on these traits due to a long-lasting consumption of the drug. Nevertheless, the loss of the effect of dextromethorphan in ants after weaning is in agreement with the usual dosage advised for adults in the notice joined to packages of this drug: 20 mg for an effect lasting 4 – 6 hours or 30 mg for an effect lasting 6 – 8 hours.³³

On the other hand, a natural product such as honey has been shown to be as useful as dextromethorphan for treating dry cough.^{27,28} Other home remedies using natural products with no action on the CNS are propolis and extracts of thyme, plantain, and green cypress. They may not be as efficient as *sensu stricto* antitussive medicines but are known to provide relief against cough.

To conclude, dextromethorphan was shown to have few side effects when tested on ants used as a biological model, the most adverse effect being that it leads to dependence. This is also known in humans. Therefore, if it is consumed at therapeutic doses during a limited time for dry cough relief and only when cough is present, it appears to be safe for humans' usage. Nevertheless, some studies showed that natural products such as honey syrup can also be useful for calming cough. It must also be recalled that cough is naturally an important vital reflex allowing clearing congested respiratory tract.

Ethical consideration and conflict of interest

We affirm having maintained the ants in the best possible environmental conditions. We also affirm having no conflict of interest as for the use of dextromethorphan. The two authors have equally and complementary contributed to the realization of the present work.

Acknowledgments

None.

Funding

None.

References

- Morice A, Kardos P. Comprehensive evidence-based review of European antitussives. *BMJ Open Respiratory Research* 2016;3(1):e000137.
- Jensen L, Rømsing J, Dalhoff K. A Danish survey of antihistamine use and poisoning patterns. *Basic Clin Pharmacol Toxicol.* 2017;120(1):64–70.
- Cantisoni C, Ricci S, Grieco T, et al. Topical promethazine side effects: Our experience and review of the literature. *Biomed Res Int.* 2013;2013:151509.
- Southard BT, Al Khalili Y, Promethazine. StatPearls Publishing LLC. 2020.
- Chiappini S, Schifano F, Corkery JM, et al. Beyond the purple drank: Study of promethazine abuse according to the European medicines agency (EMA) adverse drug reactions (ADR) reports. *J Psychopharmacology.* 2021;1–12.
- Orriols L, Gaillard J, Lapeyre-Mestre M, et al. Evaluation of abuse and dependence on drugs used for self-medication. A pharmacoepidemiological pilot study based on community pharmacies in France. *Drug Saf.* 2009;32(10):859–873.
- Eccles R, Morris S, Jawad M. Lack of effect of codeine in the treatment of cough associated with acute upper respiratory tract infection. *J Clin Pharma Ther* 1992;17(3):175–180.
- Freestone C, Eccles R. Assessment of the antitussive efficacy of codeine in cough associated with common cold. *J Pharm Pharmacol.* 1997;49(10):1045–1049.
- Sevelius H, Colmore J. Objective assessment of antitussive agents in patients with chronic cough. *J New Drugs.* 1966;6(4):216–223.
- Sevelius H, McCoy J, Colmore J. Dose response to codeine in patients with chronic cough. *J Clin Pharma Ther.* 1971;12(3):449–455.
- Aylward M, Maddock J, Davies D, et al. Dextromethorphan and codeine: Comparison of plasma kinetics and antitussive effects. *Eur J Respir Dis.* 1984;65(4):283–291.
- Matthys H, Bleicher B, Bleicher U. Dextromethorphan and codeine: Objective assessment of antitussive activity in patients with chronic cough. *J Int Med Res.* 1983;11(2):92–100.
- Smith J, Owen E, Earis J, et al. Effect of codeine on objective measurement of cough in chronic obstructive pulmonary disease. *J Allergy Clin Immunol.* 2006;117(4):831–835.
- Dicpinigaitis P, Dobkin J, Rauf K. Comparison of the antitussive effects of codeine and the GABA-agonist baclofen. *Clinical Drug Investigation.* 1997;14:326–329.
- Fuller R, Karlsson J, Choudry N, et al. Effect of inhaled and systemic opiates on responses to inhaled capsaicin in humans. *J Appl Physiol.* 1988;65(3):1125–1130.
- Dicpinigaitis P, Morice A, Birring S, et al. Antitussive drugs – Past, present and future. *Pharmacol Rev.* 2014;66(2):468–512.
- Faruqi S, Wright C, Thompson R, et al. A randomized placebo controlled trial to evaluate the effects of butamirate and dextromethorphan on capsaicin induced cough in healthy volunteers. *Br J Clin Pharmacol.* 2014;78(6):1272–1280.
- Rühle K, Criscuolo D, Dieterich H, et al. Objective evaluation of dextromethorphan and glaucine as antitussive agents. *Br J Clin Pharmacol.* 1984;17(5):521–524.
- Parvez L, Vaidya M, Sakhardande A, et al. Evaluation of antitussive agents in man. *Pulm Pharmacol.* 1996;9(5–6):299–308.
- Pavesi L, Subburaj S, Porter-Shaw K. Application and validation of a computerized cough acquisition system for objective monitoring of acute cough: A meta-analysis. *CHEST*; 2001;120(4):1121–1128.
- Tukainen H, Karttunen P, Silvasti M, et al. The treatment of acute transient cough: A placebo-controlled comparison of dextromethorphan and dextromethorphan-beta2-sympathomimetic combination. *Eur J Respir Dis.* 1986;69(2):95–99.
- Korppi M, Laurikainen K, Pietikainen M, et al. Antitussives in the treatment of acute transient cough in children. *Acta Paediatr Scand.* 1991;80(10):969–971.
- Lee P, Jawad M, Eccles R. Antitussive efficacy of dextromethorphan with acute upper respiratory tract infection. *J Pharm Pharmacol.* 2000;52(89):1137–1142.
- Bolser D. Cough suppressant and pharmacologic protussive therapy. ACCP evidence-based clinical practice guides. *Chest.* 2006;129(1 supplement):238S–249S.
- Paul I, Yoder K, Crowell K, et al. Effect of dextromethorphan, diphenhydramine, and placebo on nocturnal cough and sleep quality for coughing children and their parents. *Pediatrics.* 2004;114(1):e85–e90.
- Yoder K, Shaffer M, La Tournous S, et al. Child assessment of dextromethorphan, diphenhydramine, and placebo for nocturnal cough due to upper respiratory infection. *Clinical Pediatrics.* 2006;45(7):633–640.
- Paul I, Beiler J, McMonagle A, et al. Effect of honey, dextromethorphan, and no treatment on nocturnal cough and sleep quality for coughing children and their parents. *Arch Pediatr Adolesc Med.* 2007;161(12):1140–1146.
- Shadkam M, Mozaffari-Khosravi H, Mozayan M. A comparison of the effect of honey, dextromethorphan, and diphenhydramine on nightly cough and sleep quality in children and their parents. *J Altern Complement Med.* 2010;16(7):787–793.
- <http://reference.medscape.com/drug/balminil-dm-benylin-dm-dextromethorphan-343401>.
- Desai S, Aldea D, Daneels E, et al. Chronic addiction to dextromethorphan cough syrup: A case report. *J Am Board Fam Med.* 2006;19(3): 320–323.
- Akerman S, Hammel J, Brunette M. Dextromethorphan abuse and dependence in adolescents. *J Dual Diagn.* 2010;6(3–4):266–278.
- Martinak B, Bolis R, Black J, et al. Dextromethorphan in cough syrup: The poor man's psychosis. *Psychopharmacol Bull.* 2017;47(4): 59–63.
- Dextrométhorphane; 2021.
- Schwartz R. Adolescent abuse of dextromethorphan. *Clinical Pediatrics.* 2005;44(7):565–568.

35. Cammaerts MC. Ants as biological models for studying effects of substances used by humans. *JSM Anatomy and Physiology*. 2016;1(1):1003.
36. Cammaerts MC. Some findings on ants as models, which should be considered for caring of humans. *MOJ Biol Med*. 2017;1(5):125–128.
37. Cammaerts MC. Ants as models for examining potential adverse effects of products used by humans. *JSM Anatomy and Physiology*. 2018;3(1):1016.
38. Cammaerts MC. Brief report of the effects of seven human drugs studied on ants as models. *MOJ Biol Med*. 2019;4(2):42–47.
39. Cammaerts MC. Harmful effects of humans' environmental factors and drugs, and advices for a safer live; a study on ants as models. *World J Pharma Sci*. 2021;9(1):34–45.
40. Wehner R, Gehring W. *Biologie et physiologie animales*. Paris, Bruxelles : De Boeck Université, Thieme Verlag ; 1999.
41. Russell WMS, Burch RL. *The Principles of Humane Experimental Technique*. Johns Hopkins University; 2014.
42. Wolf FW, Heberlein U. Invertebrate models of drug abuse. *J Neurobiol*. 2003;54(1):161–178.
43. Andre RG, Wirtz RA, Das YT. Insect Models for Biomedical Research. In: Woodhead AD, editor. *Nonmammalian Animal Models for Biomedical Research*. Boca Raton, FL: CRC Press; 1989.
44. Passera L, Aron S. *Les fourmis: comportement, organisation sociale et évolution*. Ottawa, Canada: Les Presses Scientifiques du CNRC; 2005.
45. Cammaerts MC, Cammaerts D. Comparative outlook over three *Myrmica* species' biotopes and foragers' know-how. *Biologia*. 2014;69:1051–1058.
46. Cammaerts MC, Cammaerts R. The acquisition of cognitive abilities by ants: a study on three *Myrmica* species (Hymenoptera, Formicidae). *Adv Stud Biol*. 2015;7(7):335–348.
47. Cammaerts MC, Cammaerts R. Are ants (Hymenoptera, Formicidae) capable of self recognition? *J Sci*. 2015;5(7):521–532.
48. Cammaerts MC, Cammaerts R. Ants' numerosity ability defined in nine studies. *J Biol and Life Sciences*. 2020;11(1):121–142.
49. Cammaerts MC, Cammaerts R. Summary of seven more studies on numerosity abilities in an ant, four of them relating to human competence. *J Biol and Life Sciences*. 2020;11(2):296–326.
50. Cammaerts MC, Cammaerts R. Non-numerical distance and size effects in an ant. *J Biol and Life Sciences*. 2020;11(2):13–35.
51. Cammaerts MC, Cammaerts R. Weber's law applied to the ants' visual perception. *J Biol and Life Sciences*. 2020;11(2):36–61.
52. Cammaerts MC, Cammaerts R. Ants can expect the time of an event on basis of previous experiences. *ISRN Entomology*. 2016;2016:9473128.
53. Cammaerts MC, Cammaerts R. Spatial expectation of food location in an ant on basis of previous food locations (Hymenoptera, Formicidae). *J Ethol*. 2016;35(1):83–91.
54. Cammaerts MC, Cammaerts R. Ants Can Anticipate the Following Quantity in an Arithmetic Sequence. *Behav Sci*. 2021;11(2):18.
55. Cammaerts MC. Is the largely used analgesic paracetamol without any adverse effects? A study on ants as models. *EC Pharmacol Toxicol*. 2017;4(2):51–68.
56. Cammaerts MC, Cammaerts R. Ethological and physiological effects of Sativex, a cannabis-based medicine, examined on ants as models. *Acta Sci Pharm Sci*. 2020;3(8):63–84.
57. Cammaerts MC, Cammaerts R. Side effects of chloroquine and hydrochloroquine examined on ants as models. *EC pharmacol Toxicol*. 2020;8(11):57–82.
58. Siegel S, Castellan NJ. *Nonparametric statistics for the behavioural sciences*. Singapore: McGraw-Hill; 1988.
59. Cammaerts MC, Morel F, Martino F, et al. An easy and cheap software-based method to assess two-dimensional trajectories parameters. *Belg J Zool*. 2012;142(2):145–151.
60. McDonald JH. *Handbook of biological statistics*. 3rd ed. Sparky House Publishing: Baltimore, Maryland; 2014.
61. Olives T, Boley S, LeRoy J, et al. Ten years of robotripping: evidence of tolerance to dextromethorphan hydrobromide in a long-term user. *J Med Toxicol*. 2019;15(3):192–197.
62. Répertoire commenté des médicaments. Centre belge d'information pharmacothérapeutique; 2020.
63. www.vidal.fr/médicaments/gammes/dextromethorphan-biogaran-86981.html.