Acta Scientific Pharmaceutical Sciences

Volume 2 Issue 7 July 2018

Research Article

Green Clay used as a Remedy for Gastric Hyperacidity has No Harmful Effect (A Study on Ants as Models)

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Received: May 10, 2018; **Published**: June 06, 2018

Abstract

Persons suffering from heartburn commonly take care of themselves by consuming antacids such as aluminum hydroxide or a mix of calcium and magnesium carbonates. However, using ants as models, we showed that these products have several adverse effects, decreasing the ants' meat consumption, activity and cognitive abilities, as well as causing locomotion disorders, among others. Several pharmaceutical studies agree with these observations. Therefore, we looked for a safe and efficient product and thought that green clay could be such one. We thus examined the potential adverse effects of green clay, as we did for the two common antacids, and discovered that all the ants' physiological and ethological traits we examined were unchanged. However, during the first hours of their green clay consumption, the ants appeared somewhat weary, and after they stopped consuming this product, they recovered during a few hours. These two events agree with the known impact of green clay on humans, and results from its beneficial effect, i.e. it allows the organism efficiently eliminating toxic elements. Consequently, and because green clay efficiently reduces heartburn, we advise humans to use it in case of gastric hyperacidity instead of consuming aluminum hydroxide or calcium + magnesium carbonates.

Keywords: Cognition; Conditioning; Dependence; Memory; Sensitive Perception.

Abbreviations

Al: Aluminum; ang.deg.: Angular Degrees; ang.deg./cm: Angular Degrees per cm; Mm/s: Millimeter Per Second; χ^2= Chi Square; M-W: Mann-Whitney Test; vs: Versus; n°: Number; cm: Centimeter; mm: Millimeter; ml: Milliliter; µl: Micro Liter; mg: Milligram; s: second; min: Minute; h: Hours; t: Time; %: Percentage.

Introduction

Many humans suffer from gastric hyperacidity all over the word. They care of themselves by consuming antacids which are essentially aluminum hydroxide (furnished under the labels Maalox®, Gaviscon®, Contracid® and Xolaam®) as well as calcium and magnesium carbonates (furnished under the labels Rennie®, Ofloxacin®, and Magnesie Plus®). These medicines can be obtained in any drugstore without any recommendation from practitioners and are commonly reputed as being without adverse effects. However, our ethological and physiological experimentation on ants and bibliographical information about these compounds demonstrated that they are not entirely safe but presented several adverse effects. Indeed, first they decreased the ants' meat consumption, general activity, linear speed, orientation ability, trail following, audacity, tactile perception, cognition, escaping ability and conditioning capability, thus their memory. They increased their sinuosity of movement and impacted their locomotion and movement coordination. Moreover, ants did not adapt themselves to the adverse effects of these products [1,2]. Secondly, these observations on ants were in agreement with some demonstrated adverse effects on humans' health of aluminum hydroxide as well as calcium and magnesium carbonates [3-8]. Another but entirely safe product allowing efficiently treating heartburn had to be researched.

Such an efficient and safe product may be natural green clay. Indeed, this product can be used in case of infections, blood problems, skin irritation and ulceration as well as of digestive track problems, and can be used for all these health problems during three months, with a stop of a few days every three weeks. Green clay consumption may also lead, during the first few days, to the elimination of toxic, unwanted or waste elements [9-13]. On the basis of such information and having found no document relating unwanted effects of green clay, we examined, using again ants as models, if this product presents adverse effects, more precisely those we found for aluminum hydroxide as well as for calcium and magnesium carbonates.

Can ants be good biological models?

Most of physiological and ethological traits are similar for all the animals and for humans [14,15]. They are thus often first studied on animals as models, which are, for instance, fruit flies, cockroaches, bees, mice or monkeys [16]. Insects are used for their rapid development and easy maintenance in a laboratory [17]. Hymenoptera, among others, are often used [18] and ants, among them, can advantageously be used [19]. Indeed, ant colonies detain social regulation and labor division. Their members exchange information thanks to tactile and chemical signals (pheromones) produced by different glands [20-22]. They build complex nests, take care of their brood and chemically mark the different places of their environment [22]. They navigate, recruit congeners, relocate their nest, clean its inside and create cemeteries [21,22]. They detain several cognitive abilities, being able for instance to learn a behavioral sequence ([23] and references therein). All this is favorable to their use as biological models. It is what we did for examining the impact of products used by humans [e.g. 24].

How well known is the used ant species?

Ants of the genus *Myrmica* have been largely studied, among others as for their ecology, eyes morphology, angle of vision, visual perception, recruitment strategy, navigation system, learning [25], as well as the ontogenesis of some of their abilities [26]. The study of the impact of EMF on their conditioning, memory and responses to pheromones proved that they can be good biological models [27,28]. They were effectively so when examining the impact of several products used by humans [e.g. 1,2,29,30,31,32]. Effects known for humans were observed on ants and could be more accurately assessed; other effects, until then unknown and from which humans may suffer, could be revealed [30,32]. In the present work, we used again the ant *M. sabuleti* Meinert 1861 as a biological model for examining the possible adverse effects of green clay.

Which health trait can be examined on ants?

Twenty-two physiological and/or ethological traits can be examined on ants, 18 ones firstly on ants living under normal diet, then on these ants consuming a product under investigation, and 4 other traits only on ants consuming or having consumed that product. The 18 traits are: the ants' meat and sugar food consumption, general activity, speed of locomotion, sinuosity of movement, orientation ability, trail following, audacity, tactile (pain) perception, brood caring, aggressiveness towards nestmates and towards aliens (so, the ants' social relationship), cognition, escaping ability, visual and olfactory conditioning, visual and olfactory memory. The 4 other traits are: the ants' adaptation to the adverse effects of the product, habituation to its beneficial effect, dependence on its consumption, and the loss of its effects after its consumption was stopped.

Adaptation to a product occurs when its adverse effects decrease over its consumption. Habituation to a product develops when its sought-after effect decreases over its consumption. Dependence on a product appears when an individual consuming it prefers a diet including this product to a diet free of it.

Material and Methods

Collection and maintenance of ants

The experiments were conducted on two colonies of *M. sabuleti* collected in summer 2016, in an abandoned quarry located in the Ardenne region (Belgium) and on another colony of *M. sabuleti*, also collected in summer 2016 in the same locality, which allowed making the control of the conditioning experiment and which furnished the 'aliens' of the aggressiveness experiment. These colonies were maintained in cotton plugged glass tubes containing some water, and the nest tubes of each colony were deposited in a tray (34 cm x 23 cm x 4 cm) as usual [1,2]. The ants received pieces of *Tenebrio molitor* larvae (Linnaeus, 1758) three times per week, and permanently sugar water in cotton plugged small tubes. The ambient temperature equaled 19 - 21°C, the humidity was about 80%, the lighting equaled about 330 lux while working on ants and the electromagnetism was of 2 µWm^2, these conditions being optimum for the species. The ants belonging to the same colony are often here named 'nestmates', as generally do researchers on social insects.

Solution of green clay given to the ants

Humans are advised to consume, per day, a small spoon of green clay diluted in a glass of water. Humans commonly drink one liter of water per day. So, when they care of themselves using green clay, they consume a small spoon of that product together with one liter of water. Insects and thus ants, due to their excretory system and external cuticle, drink about 10 times less water than mammals. Consequently, to be under a diet similar to that of humans consuming green clay, the ants should be provided with a solution of a small spoon of green clay in 100 ml of saturated sugar water, or ½ small spoon of green clay in 50 ml of saturated sugar water. We made the latter solution and kept it at -25°C. The tap water used had a pH of 7.75. After having been defrosted and homogenized, 5 ml of the green clay solution were poured into the kind of small tube used to furnish sugar water to the ants, and such a tube was set in the tray of each colony. We checked each day if the ants drunk the sugared green clay solution and they did though being not very numerous in doing so. The cotton plug shutting the tubes was refreshed every 2 - 3 days, and the content of the tubes was renewed every week.

Preliminary remarks

The experimental designs and protocols are those previously used for examining the effects of several products consumed by humans. For avoiding a too long text as well as plagiarism, these designs and protocols are here briefly related, and the readers are advised to find details in the cited works.

All the controls were made on the ants living under normal diet. After that, a sugar water + green clay diet was given to the ants, and one day later, (if not otherwise stated), all the experiments were made again on the ants consuming green clay. Let us add that the latter experiments were made without looking to the results previously obtained (i.e. two weeks before since the 18 traits were examined in the same order during the controls and the test experiments) on ants under normal diet in order to work fully objectively.

Meat and sugar water consumption, general activity

The ants being on the sugar water, on the *T. molitor* larvae, and in activity at any place of their habitat were counted six times per day during six days, at the same times o'clock each day (Table 1, Daily counts) (as in [1,2] for example). The mean of these daily counts was established (Table 1, Daily means), and the six daily means obtained for ants consuming green clay were compared to the six daily means obtained for ants under normal diet using the non-parametric test of Wilcoxon [33]. The averages of the daily means were also established (Table 1, last line).

Linear and angular speeds, orientation to an alarm signal

The experiments were made on ants moving in their foraging area, the linear and angular speeds being assessed giving no stimulus to the ants, the orientation being assessed by presenting them with a nestmate tied to a piece of paper (Figure 1A). This nestmate emitted its attractive mandibular glands alarm pheromone. As in previous works [e.g. 31,32], for each variable, the trajectory of 40 workers (20 of colony A + 20 of colony B) was recorded on a transparent polyvinyl sheet and analyzed with appropriate software [34]. 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 given location is the sum of the successive angles made by the direction of the trajectory and that towards the location, divided by the number of measured angles. When the obtained value of orientation is lower than 90°C, the animal has a tendency to orient itself towards the location; when it is larger than 90°C, the animal has a tendency to avoid the location. The median and quartiles of each distribution of 40 values were calculated (Table 2, lines 1, 2, 3). The distributions obtained for ants consuming green clay were compared to those obtained for ants under normal diet, using the non-parametric χ^2 test [33].

Trail following

The trail pheromone of *Myrmica* ants is produced by the workers' poison gland. As in previous works [31,32], 10 poison glands were isolated into 500 µl of hexane then set for 15 minutes at -25°C. Then, 50 µl of this hexane solution was deposited, using a normograph pen, on a circumference (R = 5 cm) pencil drawn on white paper and divided into arcs of 10 angular degrees. The obtained circular trail was deposited in the ants' tray and the ants' behavior was quantified by the number of arcs of 10 angular degrees 20 ants of each colony walked along the trail (Figure 1B). The distribution of the 40 values obtained was characterized by its median and quartiles (Table 2, line 4). The values corresponding to ants consuming green clay were compared to those corresponding to ants living under normal diet using the non-parametric χ^2 test.

Audacity

As previously [31,32], a tower tied to a platform, both made of strong white paper (Steinbach®, tower height = 4 cm, tower diameter = 1.5 cm), was presented to the ants in their tray, and those moving on this apparatus were counted 10 times over 10 min (Figure 1C). The mean and the extremes of the recorded values were established (Table 2). The values obtained for the two colonies as well as in the course of two successive minutes were added (as in [1,2]), and the five sums obtained for ants consuming green clay were compared to the five sums previously obtained for these ants under normal diet using the non-parametric Wilcoxon test [33].

Tactile (pain) perception

The ants' walking on a rough substrate allows assessing their tactile (probably pain) perception. When acutely perceiving the rough character of the substrate, the ants walk slowly and sinuously. When weakly perceiving such an uncomfortable character, they walk more quickly and less sinuously. Consequently, as previously [31,32], a folded piece (3 cm x 2 + 7 + 2 = 11 cm) of emery paper n° 280 paper was tied to the borders and the bottom of a tray (15 cm x 7 cm x 4.5 cm), dividing so the tray in a first zone 3 cm long, a second zone 3 cm long containing the emery paper, and a last zone 9 cm long. Such an apparatus was constructed for each colony. For making the experiment, 12 ants of each colony were transferred into the first zone of their apparatus. When leaving this zone, they walked for a time on the emery paper. At that time, their linear and angular speeds were assessed as usually (see here above 'Linear and angular speeds') (n = 24; Table 2, line 6). The values corresponding to ants consuming green clay were compared to those corresponding to ants under normal diet using the non-parametric χ^2 test.

Brood caring

For each colony, a few larvae or nymphs were removed from the nest and deposited in front of the entrance. Each time, five of these larvae and the ants' behavior towards them were observed (Figure 1E). The larvae among the ten (5 x 2) observed and not yet replaced in the nest were counted after 5 s, 2, 4, 6, 8, and 10 minutes, and the numbers obtained for each colony were added (Table 3, line 1). The numbers corresponding to ants consuming green clay were compared to the numbers corresponding to ants under normal diet using the non-parametric Wilcoxon test [33].

Aggressiveness against nestmates and aliens

These traits were assessed in the course of ten dyadic encounters (5 for each colony) with either a nestmate or an alien ant, as in previous works [31,32]. Each encountering was conducted in a cylindrical cup (diameter = 2 cm, height = 1.6 cm), the borders of which were covered with talc. Each time, an ant of colony A or B was observed for 5 minutes, and its behavior towards the opponent assessed by the number of times it did nothing (level 0 of aggressiveness), contacted the opponent with its antennae (level 1), opened its mandibles (level 2), gripped the other ant (level 3), tried to sting or stung the other ant (level 4) (Figure 1F, 1G). The numbers obtained for the two colonies were added (Table 3, lines 2 and 3), and the results corresponding to ants consuming green clay were compared to those corresponding to ants living under normal diet using the non-parametric $\chi 2$ test. The ants' aggressive behavior was also assessed by the variable "a" = number of aggressiveness levels 2 + 3 + 4 / number of levels 0 + 1, as done in previous studies [1,2].

Cognition

This trait was examined using the protocol elaborated while studying the effects of nicotine [35]. Two pieces of white paper (Steinbach®, 12 cm x 4.5 cm), duly folded, were inserted in a tray (15 cm x 7 cm x 4.5 cm) in order to divide the tray in a first small loggia, then in a path with twists and turns, and finally in a large loggia containing a piece of wet cotton. Such an apparatus was built for each colony. For conducting an experiment, 15 ants of each colony were transferred in the first loggia of their apparatus, and those present in this loggia and in the large one were counted after 30 s, 2, 4, 6, 8, 10 and 12 min. The numbers obtained for the two colonies were added (Table 3, line 4). The results obtained for ants consuming green clay were compared to those previously obtained for ants under normal diet using the non-parametric Wilcoxon test.

Escaping ability

This trait was assessed as in previous studies [1,2]. For each colony, 6 ants were set under a reversed polyacetate glass (h = 8 cm, bottom diameter = 7 cm, ceiling diameter = 5 cm) deposited in the ants' tray. The glass presented a small notch (3 mm height, 2 mm broad) in the rim of its bottom for giving to the ants the opportunity of escaping (Figure 1H). For assessing the ants' escaping ability, those escaped and those still enclosed were counted after 30 s, 2, 4, 6, 8, 10 and 12 minutes. The results obtained for the two colonies were added (Table 3, line 5), and the sums corresponding to ants consuming green clay were compared to those corresponding to ants under normal diet using the non-parametric Wilcoxon test. The ants' ability in escaping was also assessed by the variable "n° of escaped ants after 12 minutes / 12".

Visual and olfactory conditioning and memory

After the ants had consumed green clay during 8 days, we examined if this product impacted the ants' conditioning and memorizing capabilities, using a protocol many times employed [31,32]. The control values were those previously obtained on the third collected colony [36]. A green hollow cube was set above the entrance of the sugar water tube, the ants starting so to be visually conditioned to that cue. After this experiment, pieces of basilica were deposited in front of the entrance of the sugar water tube, the ants starting then to be olfactory conditioned to that odorous cue. Tests were made over time, first while ants were expected to acquire conditioning, then, after removal of the cue, while they were expected to lose it. To make a test, ten ants of colony A and of colony B were individually set in a Y-apparatus provided with a green hollow cube or pieces of basilica in one of its branch (Figure 1I, 1J), the right or the left one, this being randomly chosen. Moving into the branch containing the cue was considered as giving the correct response. For each test, the response of the 20 used ants was recorded, and the proportion of correct responses calculated (Table 4). These proportions obtained for ants consuming green clay were compared to those previously obtained for ants under normal diet using the non-parametric Wilcoxon test.

Adaptation to adverse effects of green clay

Having finally found no adverse effect of green clay on the ants' examined traits, we could not study such an adaptation.

Habituation to beneficial effects of green clay

We found no beneficial effect of green clay on the different physiological and ethological traits we here examined, even if that product may have several favorable effects on the health in general. Habituation could thus not be examined in the present work.

Dependence on green clay consumption

We examined if ants developed some dependence on green clay consumption after they consumed this product during 9 days. The protocol was identical to that employed in previous studies [1,2,32]. For each colony, 15 ants were transferred into a tray (15 cm × 7 cm × 5 cm) in which two tubes (h = 2.5 cm, diam. = 0.5 cm) had been set, the one full of sugar water, the other one full of the sugar solution of green clay used throughout the present work (Figure 1K). The tube containing green clay was located on the right side in one tray, and on the left side in the other tray. The ants coming onto each presented liquid were counted 15 times over 15 minutes, and the counts corresponding to each kind of liquid were separately added. The counts obtained for each colony were also added. The sums were compared to the numbers expected if ants went randomly drinking each kind of provided liquid, using the non-parametric goodness of fit χ^2 test [33].

Decrease of the effect of green clay after its consumption was stopped

We detected no adverse effect as well as no beneficial one of green clay on the different physiological and ethological traits here considered. Of course, green clay may have other effects on the health, but we did not assess them. Consequently, we could not study the decrease of the impact of green clay on the health after its consumption was stopped. We could only roughly appreciate this decrease looking attentively to the ants' behavior after their weaning, and on basis of the result of the dependence experiment (see the 'Results and Discussion' section).

Results and Discussion

A First observation

During their first 12 hours under green clay diet, the ants were, at first, reluctant in coming onto the sugar water containing this product, and not very active. Nevertheless, progressively, they came drinking the sugar water containing green clay. Doing so, they seemed tired, walking slower, as if green clay acted on their physiology. After these 12 hours period, they however appeared to be in very good health, walking frankly, being numerous on the *T. molitor* larvae and very active inside their nest, taking actively care of their brood and queen. Also, they gently contacted one another with their antennae, and found very quickly their nest entrance and food sites. Let us state that, when we subsequently assessed the potential physiological and ethological effects of green clay on ants, we were not influenced by this first observation.

Food consumption, general activity

Ants consuming green clay consumed as much meat as when under normal diet (Table 1; N = 6, T = -17, +14, P = 0.280). Thus, green clay did not affect meat consumption. However, ants under green clay diet drank less sugar water than ants under normal diet (Table 1; N = 6, T = +1, -20, P = 0.031). The water solution of green clay, even if sugared, had perhaps a not pleasant taste for the ants. The obtained result probably revealed this unpleasant taste rather than some decrease of appetite. As for the ants' general activity, there was no difference at all between that of ants under green clay diet and that of ants under normal diet (Table 1; N = 6, T = -10, +11, P = 0.50). Thus, green clay did not impact the individuals' activity.

Linear and angular speed

These traits appeared to be not impacted by green clay consumption (Table 1, lines 1, 2). Indeed, the linear speed of ants consuming this product was slightly, though not statistically significantly, higher than that of ants under normal diet (χ^2 = 5.1, df = 3, 0.10 < P < 0.20), and their angular speed was a little, but not significantly, lower than that of ants under normal diet (χ^2 = 4.84, df = 3, 0.10 < P < 0.20). These numerical results were in agreement with the ants' frankly walking which was obvious to the observer.

Orientation towards an alarm signal

Ants consuming green clay oriented themselves towards an alarm signal as well as ants living under normal diet (Table 2, line 3; Figure 1A). Under green clay diet, the ants even reached more promptly the presented tied nestmates. However, the difference of orientation between ants under one and the other kind of diet was not significant (χ^2 = 2.49, df = 3, 0.30 < P < 0.50). This result was nevertheless in agreement with the observation that the ants found easier their nest entrance and food sites while consuming green clay.

Trail following

Ants under normal diet as well as those consuming green clay followed a circular trail along meanly 12 arcs of 10 ang. deg. (Table 2, line 4; Figure 1B). There was no statistical difference between the ants' response to their trail pheromone, did they consumed green clay or not (χ^2 = 0.85, df = 2, 0.50 < P < 0.70). Green clay consumption had thus no impact on this ants' physiological and ethological trait.

Audacity

While consuming green clay, the ants were slightly more inclined in coming onto the presented unknown apparatus than while living under normal diet (Table 2, line 5; Figure 1C). However, this difference of behavior was at the limit of significance (N = 4, T = +10, P = 0.06). In fact, the observation showed that green clay slightly and favorably increased the ants' foraging behavior without leading them to perform too risky behavior.

Days	Colories	Sugar water diet			Sugar water + Green clay diet		
		Meat	Sugar water	Activity	Meat	Sugar water	Activity
		Daily counts					
I	A	1 1 1 1 1 1	5 5 4 4 4 5	16 15 16 16 16 17	3 3 3 3 3 2	0 1 1 1 1 1	13 12 13 10 12 12
	B	2 2 3 3 3 3	2 1 1 2 2 2	13 14 13 14 14 15	3 3 4 3 3 2	1 1 1 1 1 1	10 10 11 12 12 13
II	A	2 2 1 2 2 2	2 2 3 2 3 3	12 11 12 13 12 13	1 1 2 1 2 1	4 4 3 3 3 4	10 9 9 12 11 12
	B	3 2 3 3 3 3	5 5 4 4 5 5	10 9 10 10 11 11	2 2 2 2 1 2	2 2 3 3 3 2	9 9 10 10 9 10
III	A	1 1 1 1 2 1	2 3 2 4 4 3	9 10 10 13 14 13	3 3 2 2 2 3	2 2 2 2 2 1	8 9 9 13 13 12
	B	1 1 1 1 0 1	2 2 2 3 3 2	8 9 9 8 9 8	1 1 0 1 2 2	1 1 0 1 1 2	8 8 9 9 10 10
IV	A	2 3 3 2 2 2	2 2 3 3 3 2	9 10 10 11 10 11	1 2 2 1 1 2	1 2 2 1 1 0	13 14 13 18 16 17
	B	2 2 1 2 1 2	2 3 3 1 2 2	10 9 10 11 12 11	1 1 2 1 1 1	1 1 1 1 1 1	13 14 14 15 16 16
V	A	1 1 0 1 1 2	5 5 4 5 5 6	10 11 10 11 11 10	2 2 1 1 1 2	1 1 2 1 2 1	14 13 13 14 14 13
	B	1 1 1 1 2 1	2 2 1 2 3 3	10 11 12 12 12 11	1 1 2 1 1 2	1 2 1 1 1 2	10 11 11 13 12 13
VI	A	1 2 1 1 2 2	1 2 2 1 1 2	9 10 10 12 13 12	0 0 0 0 0 0	2 2 3 2 2 3	16 14 14 13 14 14
	B	0 1 1 1 1 1	1 1 1 1 1 0	9 8 9 11 11 10	0 0 0 1 1 0	1 1 1 1 0 0	7 7 6 5 6 5
		Daily means					
I	A + B	1.83	3.08	14.92	2.92	0.92	11.67
II	A + B	2.33	3.58	11.17	1.58	3.00	10.00
III	A + B	1.00	2.67	10.00	1.83	1.42	9.83
IV	A + B	2.00	2.33	10.33	1.33	1.08	14.92
V	A + B	1.08	3.58	10.92	1.42	1.33	12.58
VI	A + B	1.17	1.17	10.33	0.17	1.50	10.08
		Average of daily means					
		1.57	2.74	11.28	0.53	0.92	10.08

Table 1: Effect of green clay on the ants' food consumption and activity.

Green clay did not impact the ants' meat consumption and activity. It decreased their sugar water consumption probably because clay gave an unpleasent taste to that water.

Experimental details and statistics are given in the text.

Tactile (pain) perception

This trait was not affected by green clay consumption (Table 2, lines 6, 7). The ants consuming this product perfectly perceived the rough, uncomfortable character of the provided substrate and moved on it consequently, slowly, cautiously, sinuously (Figure 1D). The difference between ants under normal diet and green clay diet as for their locomotion on a rough substrate was not significant (linear speed: $\chi^2 = 0.63$, df = 2, $0.70 < P < 0.80$; angular speed: $\chi^2 = 1.71$, df = 2, $0.30 < P < 0.50$). Thus, green clay did not affect the ants' sensitive perception.

Traits	Sugar water diet	Sugar water + Green clay diet
Linear speed (mm/s)	11.7 (10.2- 12.5)	12.0 (10.6 - 14.0)
Angular speed (ang. deg./cm)	120 (102 - 133)	103 (89 - 126)
Orientation (ang. deg.)	36.4 (26.0 - 53.2)	31.8 (23.7 - 40.9)
Trail following (n° arcs)	12.0 (8.7 - 14.0)	12.0 (8.0 - 15.3)
Audacity (n° ants)	1.50 [1 - 2]	1.75 [1 - 3]
Tactile (pain) perception:		
Linear speed (mm/s)	4.9 (4.2 - 5.5)	5.4 (4.5 - 5.9)
Angular speed (ang. deg./cm)	285 (265 - 341)	291 (250 - 325)

Table 2: Effects of green clay on six ethological and physiological traits.

Green clay did not impact any of these traits, and even somewhat improved them. More details and statistics are given in the text. mm/s: millimeter per second; ang.deg./cm: angular degrees per centimeter; ang.deg.: angular degree; n°: number.

Brood caring

Ants took care of their brood as efficiently, did they consume green clay or not (Table 3, line 1; Figure 1E). There was no statistical difference in the number of not re-entered larvae between ants under normal or green clay diet (N = 1, NS). The ants re-entered thus the larvae inside the nest within the same time period (in fact as quickly as they could) whatever their diet.

Aggressiveness against nestmates

Ants consuming green clay never aggressed their nestmates, and behaved just like ants living under normal diet, essentially touching their nestmates with their antennae or simply doing nothing (Figure 1F; Table 3, line 2). The difference, between ants under one or the other kind of diet, in the numerical values assessing this social behavior was not significant ($\chi^2 = 4.32$, df = 2, $0.20 < P < 0.30$). This result was in agreement with the previous one relative to brood caring. Thus, green clay did not impact the ants' social relationship.

Aggressiveness against aliens

Ants consuming green clay attacked alien ants in the same way (performing the same acts, with the same intensity) as those living under normal diet (Table 3, line 3; Figure 1G). The difference in the numerical values assessing such aggression, between ants consuming or not green clay was not significant ($\chi^2 = 0.73$, df = 3, $0.80 < P < 0.90$). Consequently, green clay did not impact the ants' perception of their own and of an alien colonial odor and did not affect their reaction in front of an alien odor.

Cognition

Green clay had no impact on the ants' cognitive abilities required to cross a twists and turns path (Table 3, line 4). Under normal diet, five ants among 30 could perform the task, and 14 ones were still in front of the difficult path after the 12 experimental minutes. While consuming green clay, five ants could also perform the task, and 14 ones remained also in front of the twists and turns path after the 12 experimental minutes. The difference of cognitive abilities between ants' under one or the other kind of diet was not significant (small area: N = 5, T = +11.5, P = 0.187; large area: N = 2, NS).

Escaping behavior

Green clay did not impact this ants' behavior (Table 3, last line; Figure 1H). Under normal diet, 11 ants among 12 could escape from the enclosure inside of which they had been experimentally set, and only one was thus still in this enclosure after the 12 experimental minutes. While consuming green clay, 10 ants could escape and two were still enclosed after the 12 experimental minutes. The difference of escaping capability between ants under one or the other kind of diet was not significant (concerning ants escaped as well as still enclosed: N = 4, T = -7.5, +7.5, P = 0.250). This result was in agreement with the previous one on ants' cognition and allowed presuming that green clay has no effect on the central nervous system, a presumption checked in the following experiment.

Visual and olfactory conditioning and memory

Concerning visual conditioning, no statistical difference appeared between ants under normal or green clay diet (Figure 1I; Table 4; visual conditioning). It should however be noted that ants acquired the visual conditioning score of 80% somewhat slower while consuming green clay. This can be due to the lesser consumption of sugar (see Table 1), an impact revealed in [37], this lesser consumption perhaps resulting from the unpleasant taste of the sugar water containing green clay. However, even if acquiring somewhat slowly visual conditioning, the ants consuming green clay reached a better score than ants under normal diet. Finally, there was no statistical difference of visual conditioning ability between ants living under one or the other kind of diet (N = 6, T = -12, +9, P = 0.422). After removal of the visual cue, ants consuming green clay retained nearly all their visual conditioning (losing only 5% of it) during at least 72 hours, while ants living under normal diet lose 10% of it after 48 hours (Table 4, visual memory). The difference of visual memory between ants under the two kinds of diet was statistically significant (N = 6, T = +21, P = 0.016).

As for the olfactory conditioning (Figure 1J), the same events as here above occurred. The ants consuming green clay seemed to acquire olfactory conditioning a little slower than ants living under normal diet, but finally reached an equivalent score (Table 4, olfactory conditioning). The difference of conditioning ability between ants under one and the other kinds of diet was not significant (N = 3, NS). After removal of the olfactory cue, the ants consuming green clay lose their olfactory conditioning a little slower than ants under normal diet (Table 4, olfactory memory), the difference between the two kinds of ants being at the limit of significance (N = 4, T = +10, P = 0.065).

Adaptation to adverse effects

Having found no adverse effect for green clay consumption, this trait could not be investigated.

Habituation to favorable effect of green clay

Among all the here studied physiological and ethological traits, no one was largely favorably modified. They were, all of them, simply unchanged or, apparently, very slightly ameliorated. We could thus not study the ants' habituation to these slightly favorable effects.

Traits	Sugar water diet	Sugar water + Green clay diet
Brood caring: numbers of larvae not re-entered in the nest over 10 minutes	t: 30s 2 4 6 8 10 10 8 6 4 1 0	t: 30s 2 4 6 8 10 10 8 6 4 2 0
Aggressiveness against nestmates	levels 0 1 2 3 4 var 'a' n° 42 53 15 0 0 0.15	levels 0 1 2 3 4 var 'a' n° 62 55 9 0 0 0.08
Aggressiveness against aliens	levels 0 1 2 3 4 var 'a' n° 3 15 42 63 42 8.16	levels 0 1 2 3 4 var 'a' n° 2 15 52 65 47 9.65
Cognition: ants in front of and beyond twists and turns in the course of 12 min	t n° in front n° beyond 30s 25 0 2 21 0 4 20 0 6 19 1 8 18 3 10 16 5 12 14 5	t n° in front n° beyond 30s 28 0 2 25 0 4 22 1 6 19 1 8 16 3 10 15 4 12 14 5
Escaping from an enclosure: ants in and out of the enclosure in the course of 12 min	t: 30s 2 4 6 8 10 12 n° in 12 11 9 7 5 3 1 n° out: 0 1 3 5 7 9 11 variable = 11/12 = 0.91	t: 30s 2 4 6 8 10 12 n° in: 12 10 8 6 5 3 2 n° out: 0 2 4 6 7 9 10 variable = 10/12 = 0.83

Table 3: Effect of green clay on five ethological and physiological traits.

These five traits were not at all affected by green clay consumption. Statistics and details can be found in the text. s: second; n°: number; min: minute; levels 0: doing nothing, 1: contacting the opponent with the antennae, 2: opening the mandibles, 3: gripping the opponent, 4: stinging the opponent or trying to do so.

Dependence on green clay consumption

Ants developed no dependence at all on green clay consumption (Figure 1K). In front of sugar water containing that product and sugar water free of it, 8 ants of colony A and 9 ants of colony B (so in total 17 ants) preferred the former liquid while 22 ants of colony A and 11 ants of colony B (so in total 33 ants) preferred the latter one. These total numbers (17, 33) did not statistically differ from those which should have been obtained if ants went randomly onto the two provided sugar waters (25, 25) (χ^2 = 2.01, df = 1, 0.30 < P < 0.50). Green clay induced thus no dependence. The small, not statistically significant, preference of ants for the sugar water free of green clay was in agreement with the ants' lower consumption of such sugar water containing green clay compared to their consumption of pure sugar water (see the Table 1). In the present experiment, the ants' preference of pure sugar water was not significant because the assessment lasted only 15 minutes. In the experiment on ants' sugar water consumption (Table 1), their preference for pure sugar water was significant because the assessment lasted six days.

Decrease of the effects of green clay after weaning

Having found no adverse effect and no pronounced favorable effect, we could not analyze with obvious statistical criteria the decrease of the effects of green clay on health after the consumption of this product was stopped. However, since there was no dependence on that product consumption, we could presume that the effects of green clay slowly decrease after weaning. After having finished the entire experimental work and replaced the sugar water containing green clay by pure sugar water, we observed the ants 2, 4, 6, 8, 10, 12, 14 and 24 hours later, looking to their activity, orientation to the nest entrance and the food sites, audacity, brood caring and escaping ability. We saw no difference during 12 hours; the ants went on being in very good health. After 14 hours, they began to be as before consuming green clay. After 24 hours, the ants appeared to move a little slower (and consequently more sinuously) than before consuming green clay (Table 5) (linear speed: χ^2 = 8.38, df = 2, 0.01 < P < 0.02; angular speed: χ^2 = 22.83, df = 3, P < 0.001). All happened as if ants needed to recover after having been treated with green clay, an event we also observed after ants consumed quinine [38]. Forty-eight hours after weaning, the ants seemed over this recovering period, and no difference could be any longer detected between the state they then presented and that they presented before consuming green clay. Indeed, their linear and angular speed no longer differed from the control ones (Table 5) (linear speed: $\chi^2 = 4.98$, df = 2, $0.05 < P < 0.10$; angular speed: $\chi^2 = 2.82$, df = 3, $0.30 < P < 0.50$). The recovery period was thus short, and the individuals were in perfect health just after. Consequently, we estimate that the effects of green clay slowly vanished in about 16 - 24 hours after weaning, after what the ants required a short recovery period, before being again in very good health and without being addicted to the product.

Traits time (h)	Normal diet *		Green clay diet		
	colony C	%	colony A	colony B	%
Visual conditioning					
7	7	70	7	5	60
24	8	80	7	7	70
31	8	80	7	8	75
48	8	80	9	8	85
55	8	80	8	9	85
72	8	80	10	8	90
Visual memory					
7	8	80	9	8	85
24	7	70	10	9	95
31	8	80	9	8	85
48	7	70	8	8	80
55	7	70	8	9	85
72	7	70	8	9	85
Olfactory conditioning					
7	7	70	7	6	65
24	8	80	8	7	75
31	8	80	8	8	80
48	9	90	10	8	90
55	9	90	9	10	95
72	9	90	9	9	90
Olfactory memory					
7	9	90	9	9	90
24	8	80	10	9	95
31	8	80	9	8	85
48	7	70	8	9	85
55	8	80	9	8	85
72	8	80	8	8	80

Table 4: Effect of green clay on ants' visual and olfactory conditioning ability and memory.

Green clay did not impact the ants' conditioning ability and somewhat enhanced their memory. Details and statistics can be found in the text. h: hour; %: percentage. * controls previously obtained [36].

Experimental condition	linear speed (mm/sec)	P	anguar speed (ang.deg./cm)	P
Control	11.7 (10.2 - 12.5)	-	120 (102 - 133)	-
Under green clay diet	12.0 (10.6 - 14.0)	0.10 < P < 0.20	103 (89 - 126)	0.10 < P < 0.20
24 hours after weaning	10.3 (9.4 - 11.1)	0.01 < P < 0.02	145 (136 - 172)	P < 0.001
48 hours after weaning	12.8 (11.4 - 13.9)	0.05 < P < 0.10	126 (111 - 137)	0.30 < P < 0.50

Table 5: Existence, in ants, of a recovery period, 24 hours after having stopped consuming green clay. This period was characterized by a lower activity, and thus a lower linear speed (and consequently a larger sinuosity), and lasted less than 24 hours (i.e. 48 hours after weaning, the ants' locomotion was as usual). More details and statistics are given in the text. mm/sec: millimeter per second; ang.deg./cm: angular degree per centimeter. P: statistical estimation of the difference versus control.

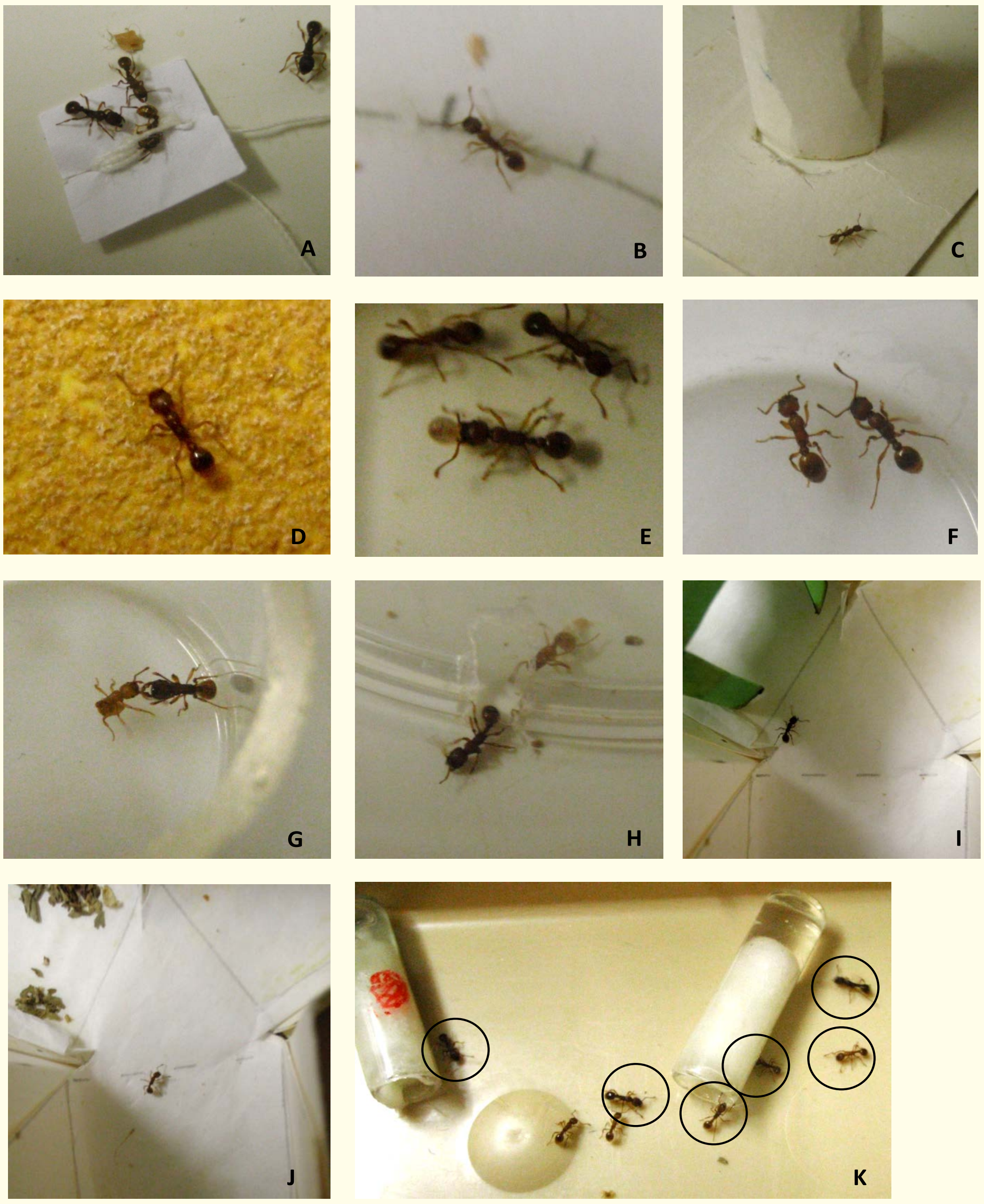

Figure 1: A few photos of ants under green clay diet. A: ants having reached a tied nestmate emitting its attractive alarm pheromone. B: an ant following a circumference on which trail pheromone had been deposited. C: an ant moving on an unknown apparatus. D: an ant moving with difficulty on a rough substrate, and slightly touching it with one antenna. E: an ant perfectly holding a larva and transporting it to the nest entrance. F: two nestmates calmly staying side by side without aggressiveness. G: an ant (on the left) trying to sting an alien ant (the darker one). H: an ant perfectly escaping from an enclosure in which it had been experimentally enclosed. I: an ant, trained to a green cube, giving the correct response in the Y-apparatus test. J: an ant, trained to basilica, reaching the choice place of the Y-apparatus, and delaying in giving the response. K: ants presented with sugar water containing green clay (red mark) and sugar water free of it; some liquid has flowed on the bottom of the tray; one ant is drinking the liquid containing green clay while five ones are drinking the liquid free of that product (black circles).

Comparison of the present results with information available on green clay

Green clay was firstly used to inhibit bacteria development [39]. It was then found being beneficial in detoxifying the body [39]. In fact, green clay attracts toxins from the blood, the tissues, the cells and the digestive track, all this being then eliminated as waste [39]. This ability of green clay in allowing the elimination of body toxins is also related in [40]. Green clay contains 60 minerals, is consequently negatively charged and binds all particles positively charged, such as bacteria, virus and toxins [40]. Russian scientists have discovered that green clay can neutralize the effects of electromagnetic radiation [40], a fact we also found 16 years ago making experiments on ants (Cammaerts, unpublished data). Another internet site [41] once more claims that green clay has a disinfecting effect on the digestive track (among others) and brings minerals to the organism, revitalizing it. In [42], all the above beneficial effects are again stated, and a risk analysis of the potential adverse effects of green clay is (at least!) reported. This analysis affirms that green clay presents no risk, no irritation, has no carcinogenic, mutagenic or neurotoxic effect, is stable, has no adverse effect at all and that no special precaution must be taken for the humans who consume it as well as for the environment [42]. This is unusual compared to what is progressively found and/or correctly divulgated about drugs or products used by humans, which have always one or the other unwanted effects on the individuals' health or on the environment. Our finding on ants that green clay has no adverse effect is thus in agreement with the observations made until now on humans by different pharmaceutics and scientific researchers. Moreover, two more observations on ants were also in agreement with those known in humans. Firstly, during the first hours of green clay consumption, the ants appeared not to be ill, but rather tired (see the first sub section of 'Results and Discussion'). Effectively, in [43] as in the directions for use joined to green clay packages, it is explained that at the beginning of clay consumption, the organism may somewhat suffer because eliminating toxins, impurities, parasites. Secondly, after weaning, we observed a short recovery period during which the ants were less active, as if they were recovering after their green clay treatment. This observation was also in agreement with the directions for use joined to green clay packages: 'stop consuming green clay for three days after having consumed it during three weeks and before beginning again consuming green clay'. Such hiatus in the course of a green clay treatment is recommended to prevent individual's tiredness caused by the elimination of toxic, unwanted and waste elements, due to the green clay effect. All our observations on ants were thus in agreement with all what is actually known on the effects of green clay on humans.

Conclusion

Heartburn is a widely spread illness which is generally treated by consuming synthesized antacids such as aluminum hydroxide and calcium + magnesium carbonates. We previously found that the two latter drugs have several adverse effects (on appetite, locomotion and cognition among others) and should not be used during long time periods. We thus examined, in the present work, in the same way we examined the two here above cited products, i.e. on ants as models, the potential adverse effects of green clay, a natural product able to efficiently reduce heartburn. We found that green clay had no adverse effect at all, and that it even somewhat enhanced the individuals' health. Therefore, we advise consuming a small amount of green clay, in water, in case of gastric and other digestive track problems, doing so according to the directions of use provided with the green clay packages. Some tiredness may occur during the first days of green clay consumption, and a three days delay of consumption should be managed every three weeks in order to recover consequently to the elimination process of toxic elements green clay induces.

Conflict of Interest

We affirm having no conflict of interest as for the use of green clay instead of the commonly used pharmaceutical antacid drugs. We make physiological and ethological researches on ants and receive no money for making so.

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Volume 2 Issue 7 July 2018