Ass. Univ. Bull. Environ. Res. Vol. 7 No. 1, March 2004

AUCES

DEVELOPMENT OF A PROPHYLACTIC METHOD AGAINST RABBIT ENTEROTOXAEMIA

Sotohy A.S. Mohamed

Dept. of Animal Hygiene, Faculty of Vet. Medicine, Assiut University

ABSTRACT :

The antimicrobial effect of tannic acid and raw material of *Acacia nilotica* fruits and leaves (tannin-rich plant) on *Clostridium perfringens* were comparatively evaluated. Different concentrations) 10 mg to 10 µg/ml)of tannic acid; fruits, and leaves were tested on *Cl. perfringens*. The obtained results revealed that the minimum inhibitory concentrations were 156, 75 and 100 µg/ml for tannic acid, fruits and leaves, respectively. Concerning "*in vivo*" experiment, 30 rabbits were randomly divided into 6 groups (5 in each) after acclimatization. Each animal of the first 5 groups received 1 ml of *Cl. perfringens* suspension per Os by stomach tube. In the next day each group was given drinking water with different tannin concentrations *ad libitum* through out the experiment. Faecal samples were collected to check the excretion rate of *Cl. perfringens*. The results revealed that, tannic acid leads to drastic reduction of *Cl. perfringens* count in the animal's gut. The excretion rate of *Cl. perfringens* was reversibly proportional to the tannic acid content of the drinking water. No significance reduction of *Cl. perfringens* count was recorded in animal's group consumed water with 0.5% tannic acid. Increasing tannic acid concentration reduced the excretion rate of *Cl. perfringens*. No *Cl. perfringens* could be detected in the faecal matter of some animals got 2% tannic acid within 1-3 weeks. Moreover, no *Cl. perfringens* could be detected in the faecal matter of most animals got 4% tannic acid after few days of treatment.

INTRODUCTION:

There is no doubt that the animal protein demand is sharply increasing all over the world in general, and in the developing countries in particular. Domestic rabbits are unequalled converter of waste food into easily digested flesh (Lotfi *et al.*, 1972). It can be kept at a comparatively low cost (Templeton, 1942 and Lotfi *et al.*, 1972). Moreover, Rabbit's meat is of high quality protein (25%), easily digested, tasty and of low fat (4%) and cholesterol (136 mg/100g) and dry matter content (Lotfi *et al.*, 1972). It is generally accepted that rabbit's meat is considered a good diet for pregnant women, children, and elderly person. Because of these peculiarities, breeding domestic rabbits can overcome present shortage of meat for human needs.

***Clostridium perfringens* is found in the alimentary tract of nearly all species of worm-blooded animals as a part of the normal intestinal flora of healthy animals (Gillespie & Timoney, 1981). Toxigenic strains are concerned in fatal toxaemias in a variety of animal species including lambs and calves (Smith, 1957), sheep and goats (Oxer, 1956), foals (Leader, 1982) and rabbits (Parish, 1961; Al-Sheikhly & Truscott, 1977; Patton *et al.*, 1978; Baskerville *et al.*, 1980; Eaton & Fernie, 1980 and Seifert *et al.*, 1996).**

Under conditions resulting from incorrect feeding (high starch, high protein and low fiber), exterior stress, and destruction of other competing natural bacteria, Clostridium proliferates secreting a diarrhea forming toxins (Fatou-Rakotobe, 1996 and Baker, 1998). Young rabbits are particularly susceptible to enterotoxaemia, especially during the 6-8 week age period. Weaning is the critical period in rabbit's life when they become susceptible to diarrhoeal disturbances from ingested bacteria that are able to colonize the digestive system. Young rabbits have a poor ability to digest starch before age of 8 weeks, so large amount of starch is passing undigested through the caecum where it can be fermented by some bacteria (Carman & Borriello, 1982; Carman & Borriello 1984 and Mackintosch *et al*., 2002). The disease has a rapid onset with death within 12-48 hours once diarrhea has been noticed (Baker, 1998). Rabbits may recover from their first episode of diarrhea only to come down with the same symptoms later or fail to thrive and reach market weight.

Plants and their extracts have been used for centuries in curing many diseases. Vegetable tannins (phenolic compounds) exhibit strong bactericidal effect on pathogenic bacteria (Henis *et al*., 1964; Schragle, 1990 and Nakahara *et al*., 1993). There are many Egyptian tannin-rich plants exhibit bactericidal effect (Megalla *et al*., 1980 and Sotohy *et al*., 1995). Sotohy (1994) found that *Acacia nilotica* is containing 35.5 and 34.0% total soluble phenols in the fruits and leaves, respectively. Moreover, *Acacia nilotica* is containing 2.96 and 0.5% condensed tannins in the fruits and leaves, respectively.

The aim of this work is to study antibacterial properties of tannic acid as well as some tannin-containing plants on the *Clostridium perfringens*, the causative agent of rabbit enterotoxaemia.

MATERIALS AND METHODS:

Bacterial species:

***Clostridium perfringens* type D (NTCC 8580) was used in this study. The organism was maintained on blood dextrose agar slants at –80 ºC. *Clostridium perfringens* cells are thawed and sub-cultured anaerobically for 24 h on blood dextrose agar medium at 37ºC. Next, some colonies were picked and homogenized in 20 ml sterile saline (0.85% NaCl, w/v). Sterile glass beads (3mm diameter) were added and shaked for 15 minutes on 250-units/minute rotatory plateform shakers.**

Plant materials:

Leaves and fruits of *Acacia nilotica* were collected, air dried and well grinded. The materials were kept in tightly closed glass containers for the next use. Phenolic compounds were determined in the *A. nilotica* by usinf Folin-Ciocalteu reagent (Makkar *et al*., 1993). On the other hand, condensed tannins were determined by proanthocyanidin assay by using butanol-HCl-Fe3+ reagent (Porter *et al*., 1986).

3-commercial tannins (sigma Aldrich, Tannin, Gallotannin)

In-vitro Study:

The minimum inhibitory concentration (MIC) required for complete destruction of the organisms was determined. Different concent-rations (10 mg/ml to 10 µg/ml) were prepared from the tannic acid as well as the raw plant materials in sterile physiological saline. The total colony count (TCC) was carried out after adding bacterial suspension (1mL) as well as after one hour (Cruicckshank *et al*., 1980, and Baily & Scott, 1994). As a control, the TCC of *Clostridium perfringens* was also conducted in sterile physiological saline.

In-vivo study:

Animals:

A total of thirty rabbits (four weeks of age) were kept one week for acclimatization. The routine clinical examination was carried out to insure their soundness. The animals were randomly divided into 6 groups (5 per each).

Experimental design:

Before starting the experiment, faecal samples were collected from each animal in sterile plastic bag to check the total *Cl. perfringens* count. Each animal of the first five groups received 1 ml of *Cl. perfringens* suspension orally using stomach tube. The last group was left as a control. Tannic acid was dissolved in drinking water with the appropriate concentration and given to the animals *ad-libitum* 24 h after infection and left through out the experiment as following:-

Group A: received 5 mg/ml.

Group B: received 10 mg/ml.

Group C: received 20 mg/ml.

Group D: received 40 mg/ml.

Group E: infected but not treated with tannic acid.

Group F: not infected, not treated.

At different time points (1, 7, 21, and 30 days), faecal samples were collected for total clostridial count.

Counting of *Cl. Perfringens:* The total colony count of *Cl. perfringens* was conduced by pour plate technique using blood glucose agar medium Cruickshank *et al*., 1980, and Toply & Wilson, 1990). The inoculated plates were anaerobically incubated at 39ºC for 48 hours. Obtained data were statistically analyzed according to Snedecor & Cochran (1989).

RESULTS :

Table (1): Effect of Tannic acid on *Clostridium perfringens.*

Tannic acid /ml	Time	
	T_0	T_{60}
10.0 mg	0	0
5.00 mg	0	0
2.50 mg	0	0
1.25 mg	0	0
0.63 mg	0	0
312.5 µg	0	0
156.3 µg	$1.8x10^3$	0
78.13 µg	$2.5x10^4$	$9.1x10^2$
39.1 µg	$2.7x10^5$	$7.2x10^3$
19.5 µg	$34x10^5$	$2.2x10^4$
9.78 µg	$1.5x10^7$	$6.8x10^4$
Control	3.5x107	2.3x107

Table (2): Effect of row *A. nilotica* leaves and fruits on *Cl. perfringens*

Tannic acid (µg/mL)	Time (min)			
	0		60	
	A.nilotica F	*A.nilotica L*	*A.nilotica F*	*A.nilotica L*
600	0	0	0	0
500	0	0	0	0
400	0	0	0	0
300	0	0	0	0
200	0	0	0	0
100	0	0	0	0
75	$4.3x10^2$	$5.2x10^5$	0	$3.7x10^4$
50	$2.3x10^4$	$8.4x10^5$	$4.2x10^2$	$2.5x10^5$
25	$5.4x10^5$	$1.8x10^6$	$3.7x10^5$	$3.3x10^5$
20	$6.7x10^5$	$2.7x10^6$	$4.2x10^5$	$1.8x10^6$
Control	$5.4x10^7$	$5.9x10^7$	$2.8x10^7$	$5.9x10^7$

F, fruits; L, leaves

Table (3): Total Clostridial count in faecal samples

Group	Count	*Cl.perfringens* count/g				
		Before the exp.	After infection & treatment/day			
			1	7	21	30
A	Min.	$1.2x10^2$	$2.8x10^2$	1.8x10	1.6x10	1.4x10
	Max.	$8.4x10^3$	$1.0x10^4$	$8.0x10^3$	$6.4x10^2$	$1.8x10^3$
	Mean	$3.3x10^3 \pm 3.2x10^3$	$4.1x10^3 \pm 4.0x10^3$	$1.9x10^3 \pm 3.4x10^3$	$2.3x10^2 \pm 2.8x10^2$	$7.0x10^2 \pm 7.6x10^2$
B	Min.	4.2x10	$1.6x10^2$	1.9x10	1.2x10	0
	Max.	$1.2x10^4$	$1.0x10^4$	$1.2x10^3$	$3.8x10^3$	$3.2x10^2$
	Mean	$2.9x10^3 \pm 5.1x10^3$	$4.1x10^3 \pm 4.6x10^3$	$3.9x10^2 \pm 4.7x10^2$	$9.1x10^2 \pm 1.6x10^3$	$9.4x10 \pm 1.3x10^2$
C	Min.	2.1x10	1.8x10	0	0	0
	Max.	$1.6x10^4$	$4.8x10^3$	$2.3x10^2$	2.8x10	1.8x10
	Mean	$4.4x10^3 \pm 6.8x10^3$	$1.7x10^3 \pm 2.3x10^3$	$5.7x10 \pm 9.7x10$	$0.6x10 \pm 1.2x10$	$0.4x10 \pm 0.7x10$
D	Min.	1.1x10	2.8x10	0	0	0
	Max.	$1.2x10^4$	$2.0x10^3$	3.2x10	1.1x10	0.5x10
	Mean	$3.4x10^3 \pm 5.0x10^3$	$5.4x10^2 \pm 8.3x10^3$	$0.6x10 \pm 1.4x10$	$0.4x10 \pm 0.5x10$	$0.1x10 \pm 0.2x10$
E	Min.	1.1x10	$2.8x10^2$	$1.2x10^2$	1.8x10	4.8x10
	Max.	$3.6x10^4$	$6.8x10^4$	$4.2x10^4$	$3.6x10^4$	$3.2x10^4$
	Mean	$8.1x10^3 \pm 1.5x10^4$	$2.4x10^2 \pm 3.2x10^4$	$1.9x10^2 \pm 2.0x10^4$	$1.4x10 \pm 1.5x10^4$	$0.8x10 \pm 1.3x10^3$
F	Min.	2.8x10	2.1x10	$1.2x10^2$	$2.8x10^2$	1.8x10
	Max.	$2.1x10^4$	$4.8x10^3$	$6.0x10^3$	$4.0x10^3$	$3.6x10^3$
	Mean	$5.5x10^3 \pm 8.8x10^3$	$2.2x10^3 \pm 2.4x10^3$	$2.3x10^3 \pm 2.3x10^3$	$1.8x10^3 \pm 1.4x10^3$	$1.9x10^3 \pm 1.4x10^3$

A, Animals received 0.5% tannic acid; B, Animals received 1% tannic acid; C, Animals received 2% tannic acid; D, Animals received 4% tannic acid; E, Animals not infected & treated; F, Animals not infected and not treated.

A: Animals treated with 0.5% tannic acid.
B: Animals treated with 1.0% tannic acid.
C: Animals treated with 2.0% tannic acid.
D: Animals treated with 4.0% tannic acid.
E: Animals infected but not treated.
F: Animals neither infected, nor treated.

DISCUSSION:

An understanding of the causes of enterotoxaemia is essential to adopting successful methods of treatment, prevention and control. As the causative agent involved is sometimes present in the hindgut (caecum) of normal rabbits, its reduction in animal's gut seems to be the key for economical control of the disease. Using highly effective, safe and cheap source is the main goal of the current study.

Results in table (1) showed that the MIC of tannic acid on *Cl. perfringens* is 156 µg/ml. No viable clostridia could be detected within few minutes after addition higher tannic acid to the bacterial suspension. On the other hand, row *A. nilotica* leaves or fruits posses higher antimicrobial properties against *Cl. perfringens*. Table (2) showed that the MICs were 75 µg/ml and 100 µg/ml for fruits and leaves, respectively. The higher bactericidal effect of row *A. nilotica* on *Cl. perfringens* was attributed to condensed tannins Takechi *et al.*, 1985). Sotohy (1994) recorded that although *A. nilotica* fruits and leaves have more or less the same total soluble phenols (34-36%), condensed tannins content in the fruits is 6 folds higher than that of the leaves.

The antimicrobial effect of tannins is due to their ability to form complexes with proteins and other polymeric substances. Not like tannic acid, condensed tannins have greater affinity for proteins due to strong hydrogen bond affinity of its carbonyl oxygen to the peptide groups of proteins (McLeod, 1974).

Tannins are seldom considered as metabolic toxins because they only act within the animal's digestive tract. The counter defense available in most herbivores is limitation of their tannin's intake below some threshold. All animals including monogastric ones could tolerate up to 5% tannins in their rations (Kibon & Maina, 1993).

Concerning the *in-vivo* study, results in table (3), revealed that the total *Cl. perfringens* was drastically reduced over the time by increasing tannic acid concentration. At 0.5%, no significant reduction in the total viable clostridia could be detected and the count was fluctuated within the normal range through out the experiment. On the other hand, the count was reduced from 2.9 $x10^3$ to 9.4x10/g after four weeks in the animal's group got 1% tannic acid. Although the count is still lower than the initial, *Cl. perfringens* count is increased in the third week. This could be attributed to the animal adaptation's trial to the new food by increasing secretion of mucosal proteins, which bound to tannins and reduce their availability (Provenza & Malechek, 1984).

By increasing tannic acid concentration in the drinking water, the total *Cl. perfringens* count was drastically reduced and no clostrdia could be detected in the faecal matter of some animals received 2% within 2-3 weeks. Moreover, no *Cl. perfringens* could be detected in most animals received 2% tannins after 4 weeks. Moreover, no viable clostridia could be detected after one week in almost all animal's group treated with 4% tannic acid (Table 3 and figure1). Muller *et al.* (1993) and Sotohy (1994) found that the excretion rate of faecal clostridia of sheep fed certain tannin-containing plants was drastically reduced by feeding animals some tannin-containing plants.

Data obtained from the *in-vivo* experiment revealed that reduction of clostridia was not as high as that recorded in the *in-vitro* study where $3.5x10^7$ viable cells of *Cl. perfringens* was completely destroyed by as low as 156 µg/ml (Table 1). This could be easily attributed to presence of large number of bacterial species of different responses to tannins as well as presence of huge amounts of protein and unsaturated lipids in the gastrointestinal tract of

plant or animal origin. All these proteins and other macromolecules could react non-specifically with the available tannins and mitigate their effect on the intestinal micro-organisms (Clark & Reid, 1974; Dugan, 1976; Jones & Mangan, 1977, and Austin *et al*., 1989). On the other hand, tannins may undergo some partial degradation in the gastrointestinal tract (Krumholz & Bryat, 1986 and Osawa, 1990).

From the obtained results, one can safely conclude that, tannin-containing plants could be used for adopting successful methods of prevention and control of rabbit enterotoxamia based on reduction of the causative agent that are able to colonize the hind gut.

REFERENCES :

Al-sheikhly, F., and Truscott, R.B. (1977): The inactivation of Clostridium perfringens and its toxins in the production of necrotic entritis of chickens. Avian diseases, 21: 256-263.

Austin, P.J.; Suchar, L.A.; Robbins, C.T. , and Hagerman, A.E. (1989): Tannin-binding proteins in saliva of deer and their absence in saliva of sheep and cattle. J. chem.. Ecol., 15: 1335-1339.

Babiker, S.H. (1996): Production of Vet. Vaccines in the Sudan. Ber. Munch. Tieraerztl. Woschen., 109: 8-9.

Baily, W.R., and Scott, E.G. (1994): Diagnostic microbiology. 9th Ed. The C.V.Mosby, Saint Louis.

Baker, D.G. (1998): Natural pathogensis of laboratory mice, rats, and rabbits and their effects on research. Clin. Microbiology. Rev., 11: 231-266.

Baskerville, M.; Wood, M., and Seamer, J.H. (1980): Cl. perfringens type E enterotoxaemia in rabbits. Vet. Rec., 107 (1): 18-19.

Carman, R.J. and Borriello, S.P. (1982): Clostridium spiroforme isolated from rabbits with diarrhoea. Vet. Rec., 111: 461-462.

Carman, R.J. and Borriello, S.P. (1984):Infections nature of *Cl.spiroforme*-mediated rabbit enterotoxaemia. Vet. Microbiol., 9: 497-502.

Clark, R.T.J. and Reid, C.S.W. (1974): "Foamy bloat of cattle" A review. J. dairy cattle, 57:753-785.

Cruickshank, R.; Dugid, I.P.; Mormion, B.P., and Swain, R.H. (1980): Medical Microbiology, 12th Ed., Vol. 11, reprinted Churhill Livingstone and Robert Stevenson Endinburg EHI 3 HF.

Dugan, L.J. (1976): "Lipids" In: Principle of food science part1; food chemistry, (Ed. Fennena, O.R., pp. 139. Marcel, Dekker, Inc., New York).

Eaton, P., and Fernie, D.S. (1980): Enterotoxaemia invloving Cl. perfringens iota toxin in a hysterectomy-derived rabbits colony. Lab. Animals, 14 (1): 347-351.

Fatou-Rakotobe, J., anmdRajanarison, J. (1996): Technologie der Herstellung von Anigenen gegenBodenseuchen in Madagasker. Br. Munch. Tieraerztl. Woschr., 109: 6-7.

Gillespy, J.H., and Timoney, J.F. (1981): Infectious diseases of domestic animals. Comstock publishing Associates, Cornell University press, Ithaca and London.

Henis, Y.; Tagari, H., and Volcani, R. (1964): Effect of water extracts of carob pods, tannic acid and their derivatives on the morphology and growth of microorganisms. Water microbial., 12 (3): 201-209.

Jones, W.T., and Mangan, J.L. (1977): Complexes of condensed tannins of

sainfoin (Onobrychis viciifolia scop.) with fraction of leaf protein and with submaxillary mucoprotein and their reversal by polyethylene glycol and pH. J.Sci. food Agric., 28: 126-136.

Kibon,A., and Maina,A.H.B. (1993): Dry Acacia sieberiana pods as a supplement to a low quality forgae for growing lambs in northern Nigeria. Trop. Anim. Health and production, 25: 59-64.

Krumholz, L.R., and Bryant, M.P. (1986): Eubacterium oxidoreducens species b requiring H2 or formate to degrade gallate, pyrogallol, phloroglucinol and quercetin. Arch. Microbial., 144: 8-14.

Leader, G.H. (1982): The clinical aspects of some diseases met within young throughbred foals. Vet. Record, 64: 241-245.

Lotfi, A.Y.,; Hassan, A.H., andIsmail, A.M. (1972): Studies on the value of rabbits and flesh producing animals. Egyptian Vet. Med. J., XX (20): 143-147.

Mackintosch, C.; Haigh, J.C., and Griffin, F. (2002): Bacterial diseases of formed deer and bison. Rev. Sci. Tech. Off. Int. Epiz., 21 (2): 249-263.

Makkar, H.P.S.; Blummel, M.; Browy, N., and Becker, K. (1993): Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J.Sci. food agriculture, 61: 161-165.

McLeod, M.N. (1974): Plant tannins-their role in forgae quality. Nutr. Abstr., 44: 803-815.

Megalla, S.E.; El-Keltawi, N.E.M., and Ross, S.A. (1980): A study of antimicrobial action of some essential oil constituents. Berba polonica, 26 (3): 181-186.

Mueller, W.; Bauman, M., and Younan, M. (1993): Use of tropical plant polyphenols as a feed additive with respect to pathogenic clostridia species. 3rd Int. sheep Vet. Conf., Vol., 17 pp.232.

Nekahara, K.; Kawabata, S.; Hiroyuli, O.; Ogura, K.; Tanaka, T.; Ooshima, T., and Hamada, S. (1993): Inhibitory effect of Oolong tea polyphenols on glucosyl-tranferase of mutans Streptococci. Appl. & Environ. Microbiol., 59 (4): 968-973.

Osawa, R. (1990): Formation of a clear zone of tannin-treated brain heart infusion agar by a Streptococcus species isolated from faeces of koalas. Appl. & Environ. Microbiol., 56 (3): 829-831.

Oxer, D.T. (1956): Enterotoxaemia in goats. Australian Vet. J., 32: 62-66.

Parish, W.E. (1961): Necrotic enteritis in the fowl. III the experimental disease. Journal of comparative pathology and therapeutics, 71: 405-413.

Patton, N.M.; Holes, H.T.; Riggs, R.J., and Checke, P.R. (1978): Enterotoxaemia in rabbits. Lab. Animals Sci., 28(5): 356-340.

Porter, L.J.; Hrstich, L.H., and Chan, B.G. (1986): The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochem., 25: 79-103.

Provenza, F.D., and Malechek, J.C. (1984): Diet selection by domestic goats in relation to blackburch twig chemistry. J. Applied Ecolo., 21: 831-841.

Schragle, R. (1990): Untersuchungen uber den Einfluss pflanzlicher Tannine auf die Tenazitat von pathogenen Bacterien in einem *in-vitro* pansensystem. Diss. Fak. IV, Agrawiss.II, Universitat Hohenheim, Stuttgart, Germany.

Seifert, H.S.H.; Bader, K.; Cyplik, J.; Gonzalez Salinas, J.; Roth, F.; Salinas Malendez, J.A. and Sukop, U. (1996): Environment, incidence, aetiology and immunopro-

phylaxis of soil-borne disease in north – East Mexico. J. Vet. Med., B 43 (1): 281-287.

Smith, L.D. (1957): Clostridial diseases of animals. Advance Vet. Sci., 3: 465-524.

Snedecor, G.W., and Cochran, W.G. (1989): Statistical methods. 8th Ed. Iowa State University, Press, Ames, Iowa.

Sotohy A.S. (1994): The effect of some tannin containing plants from Upper Egypt on selected pathogenic microorganisms of intestine of small ruminants. Ph.D., Fac. Of Vet. Med., Assiut University, Assiut-Egypt.

Sotohy. A.S.; Ismail, A.A., and Mueller, W. (1995): Further studies on the antimicrobial properties of some plan materials in relation to their tannin contant. 3rd Sci. Cong., Egyptian Society for cattle diseases, 3-5 Dec., 1995, Assiut-Egypt.

Takechi, M.; Tanaka, Y.; Takehara, M.; Nonaka, G., and Nishioka, I. (1985): Structure and antiherpetic activity among the tannins. Phytochem., 24:2245-2250.

Templeton, G.S. (1942): Domestic rabbits in the food for freedom programme, U.S. Dept. interior, fish and wildlife serve., wildlife leafleat No. 218, pp. 9. Nutrition Abstracts and reviews 1943 Vol. 18.

Toply, W.W., and Wilson, G.S. (1990): Principles of bacteriology, Virology and immunology. 8th. Ed., Systematic bacterilogy.

استحداث طريقة غير تقليدية للوقاية من التسمم المعوي فى الأرانب

سطوحى أحمد سطوحى

قسم الصحة– كلية الطب البيطرى – جامعة أسيوط

تم فى هذا البحث دراسة التأثير المثبط لحمض التانيك وأيضاً مطحون ثمار وأوراق نبات السنط على ميكروب الكلوستريديوم برفرنجنز . أظهرت النتائج أن تركيز ١٥٦ ميكروجرام/ملي من الحمض كانت كافيه لقتل كل الميكروبات فى خلال ساعة تقريبا من ناحية أخري وجد أن ٧٥ ميكروجرام/ملي من الثمار و ١٠٠ ميكرو جرام/ملي من الأوراق كانت كافيه لقتل الميكروبات فى خلال نفس المدة. أظهرت النتائج أن هناك تناسبا عكسيا بين كميه المادة المضافة وعدد الميكروب في براز الحيوان. هذه النتائج تثبت أن أضافه ثمار أو أوراق نبات السنط بكميات قليلة إلى علائق هذه الحيوانات يؤدي إلى انخفاض عدد الميكروبات المرضية فى معي الحيوان مما يؤدى إلى حماية الحيوان من مرض التسمم المعوي في هذا الخصوص وجد أن إضافة حامض التانيك بنسبه ٢% كانت كافيه لهذا الغرض.