

Effect of cinnamic aldehyde as repellent on behaviour and gastrointestinal tract of house rat, *Rattus rattus*

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Cinnamic aldehyde (CA) is a major constituent of cinnamomum bark oil. Though there are reports on the antifeedant effect of CA in house rat, understanding its effect on behaviour and gastrointestinal tract of house rat is necessary to develop an effective formulation for its field application against commensal rodents. In the present investigation, rats were fed on plain bait and 5% CA treated bait both under no-choice and bi-choice conditions for 30 days. Results revealed its potential as an antifeedant against *R. rattus* under both no-choice and bi-choice conditions. However, antifeedant/secondary repellent effect of 5% CA was more under bi-choice conditions. Studies on feeding and tactile behaviour of rats in response to 5% CA also revealed its irritant effect. Histomorphological studies on stomach and intestine of 5% CA treated rats revealed that in no-choice group of rats, 5% CA caused hyperplasia of non-glandular stomach and also caused reduction in number of cells of both intestinal and gastric glands which might have caused reduction in secretion of digestive juices and hormones resulting in reduction in consumption of treated bait. But in bi-choice group, GI tract was not affected. Reduction in consumption of treated bait in bi-choice group of rats might have occurred due to its irritant effect on skin and eyes of rats causing rats to prefer alternate plain bait under bi-choice conditions.

Keywords: Antifeedant, Cinnamomum bark oil, Commensal pests

Rodents are recognized as the most destructive pests due to the damage they cause to the standing crops and stored food¹⁻³. The common commensal pests are *Rattus rattus* and *Mus musculus* throughout India including the islands. They are responsible for causing huge losses in grain stores and poultry farms. The available rodent control options can be grouped into two basic approaches viz. lethal or reductional and non-lethal or preventive. The lethal approach, particularly the use of rodenticides and trapping, which provides an immediate solution to the problem, is often considered the most practical method of combating rodents while non-lethal or preventive measures involving environmental sanitation, rodent proofing, plant based repellents and antifertility agents, which may produce a more lasting effect, are rarely adopted⁴.

Many essential oils comprising volatile lipophilic components extracted from plants possess repellent properties⁵. These oils are being tried as potential candidates for weed⁶, pest and disease⁷ management. Plant essential oils, such as eucalyptus oil^{8,9} and

citronella oil¹⁰ have also been reported as repellents against rodents.

Many cinnamomum species are also rich in essential oils and tannins. Various cinnamomum bark derived compounds are cinnamyl alcohol, trans-cinnamic acid, eugenol, salicylaldehyde and cinnamic aldehyde¹¹. Cinnamic aldehyde constitutes 68% cinnamomum bark oil, is a powerful irritant and can cause stomatitis and cheilitis¹². Antignawing activity of cinnamic aldehyde is also reported against mice¹³. The combined contact, fumigant and antifeedant properties of cinnamic aldehyde make it a potentially useful grain protectant¹⁴. Earlier study in our laboratory based on consumption of 1, 2.5 and 5% CA mixed bait suggested that 5% CA has potential as an antifeedant against *R. rattus* and rats were not habituated to the treated bait¹⁵, but further detailed studies are required to determine the mode of action of cinnamic aldehyde (CA) in house rats so as to develop a relevant formulation for field application against commensal rodents.

Materials and Methods

The present work was carried out at Animal House Laboratory and Rodent Research Laboratory, Dept. of Zoology, Punjab Agricultural University (PAU),

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Ludhiana located at an intersection of 30°55' N parallel of latitude and 75°54'E line of longitude.

Collection and maintenance of animals

For present studies, the house rat, *R. rattus* of both sexes were trapped from store houses, grocery shops and poultry farms. In laboratory, rats were acclimatized before the commencement of experiment with food and water provided *ad libitum*. Food provided was prepared by mixing cracked wheat, powdered sugar and groundnut oil (WSO bait) in ratio 96:2:2. The metallic trays were kept under each cage for the collection and disposal of urine and faeces and for collection of spilled food. After acclimatization, healthy rats were weighed and grouped for experimentation. Animals were used and maintained as per the guidelines of Institutional Animal Ethics Committee. Approval of Institutional Animal Ethics Committee Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana was obtained for the usage of animals.

Effect of 5% cinnamic aldehyde on food consumption

Consumption of 5% CA treated bait by *R. rattus* was recorded both in no-choice and bi-choice feeding tests. Treated bait was prepared by dissolving the known volume of cinnamic aldehyde in known volume of methanol which was then mixed in known quantity of plain WSO bait to prepare 5% CA mixed bait. Rats weighing about 135-200 g were divided into three groups of 10 rats (5 of each sex). Before treatment, rats of treated (two groups) and untreated groups were fed on plain WSO bait for five consecutive days. During treatment, rats of treated groups were fed on 5% CA mixed bait both in no-choice and bi-choice feeding tests for 30 days. The positions of the bowls containing plain and treated WSO baits in bi-choice feeding test were changed daily to counteract the positional preferences. After treatment, consumption of plain WSO bait was recorded again for five consecutive days to determine the effect of treatment on post-treatment bait consumption. Simultaneously, rats of untreated group were fed on plain WSO bait for 30 days. Daily bait consumption of both treated and untreated baits was calculated. From daily bait consumption, antifeedant index was calculated¹⁶ both in no-choice and bi-choice feeding tests

Feeding and tactile behaviour of *R. rattus* in response to 5% CA in feed scale consumption monitor

Feeding behaviour of rats (n=3 of each sex) in response to 5% CA was recorded using feed scale consumption monitor as previously described¹⁵.

Quantitative data on feeding bout events/h, feeding bout time (s)/h and feed consumed (g)/h was recorded. Changes in tactile behavior of rats exposed to 5% CA bait were also recorded by observing the rats kept in chambers of feed scale consumption monitor.

Effect of 5% CA on gastrointestinal tract

Rats fed on 5% cinnamic aldehyde bait for 30 days both in no-choice and bi-choice feeding tests and untreated rats were autopsied to record the effect of treatment on stomach and intestine. Stomach and intestine from both treated and untreated rats were collected, washed in 0.9% saline and their morphometric measurements were taken. Anterior, middle and posterior portion each of stomach and small intestine were fixed in 10% neutral buffered formalin for 48 h and processed using a standard histological procedure¹⁷. The tissue sections were then evaluated for histomorphometric and histological changes under compound light microscope and photomicrographs were taken using digital camera.

Four layers of the wall of the stomach i.e. mucosa, submucosa, muscularis externa and serosa were identified¹⁸. Thickness of mucosa, submucosa, muscularis externa and major and minor diameter of different type of cells present in mucosa of stomach were measured using ocular micrometer having arbitrary graduation. Histologically, stomach of rat is divided into glandular and non-glandular regions. Cells of stratified squamous epithelium of non-glandular stomach and columnar cells of epithelial layer/mucosal cells as well as chief cells, parietal cells, neuroendocrine cells and stem cells of gastric glands were identified¹⁹ and counted.

Four layers of the wall of small intestine i.e. serosa, muscularis externa, submucosa and mucosa were identified²⁰. Major and minor diameter of small intestine and its lumen, thickness of mucosa, submucosa, muscularis externa and major and minor diameter of different type of cells present in mucosa of small intestine were measured using ocular micrometer having arbitrary graduation. Cells of small intestinal mucosa were also identified and counted¹⁸.

The actual mean number counted of almost round cells in the stomach and intestine was corrected by a modification of Abercrombie's formula.

True count = (Observed count) × Section thickness / (Section thickness + $\sqrt{(\text{Av.dia.})^2 - (\text{Av.dia.})^2}$ 2 4

The arbitrary graduation of the ocular micrometer was calibrated using a stage micrometer by

superimposing the two scales. Calibration of ocular micrometre was done under different magnifications.

Statistical analyses

Values were determined as mean $\pm$ SE. Significance of differences were determined at 5% level of significance. Significance of difference among various parameters was determined by factorial completely randomised designs (fCRD). The statistical software used was CPCS1.

Results and Discussion

Effect of 5% cinnamic aldehyde as an antifeedant in no-choice and bi-choice feeding tests

Average consumption (g/100 g body wt.) of treated bait during treatment period was found to be significantly ($P \leq 0.05$) low as compared to plain/untreated bait under both no-choice and bi-choice conditions indicating antifeedant effect of 5% CA. However, there was a non-significant difference in antifeedant index from days 1-5 to days 26-30 during treatment period indicating antifeedant effect of 5% CA developed in treated rats (both and female) on first day of feeding which retained till day 30th of treatment period indicating rats do not develop habituation against CA as antifeedant effect persisted in rats even when they were fed on 5% CA treated bait continuously for 30 days both in no-choice and bi-choice feeding tests. However, there was a significant difference ($P \leq 0.05$) in antifeedant index between no-choice and bi-choice feeding tests (Fig. 1). Earlier studies reported antignawing and antifeedant effect of 5% CA against mice and *R. rattus*, respectively^{13,15}. Rats were not habituated to

5% CA throughout the experimental period of 27 days¹⁵. In bi-choice feeding test, antifeedant effect was more as compared to that in no-choice feeding test clearly indicating that 5% CA would be more effective as antifeedant against rodents if rats are provided with alternative food sources. Attenuation of conditioned taste aversion is slower under the choice feeding tests. This may be due to the constant presence of alternate safe food, which leads the animal to sample treated food less frequently. It is generally accepted that choice feeding test is more sensitive for detecting an aversion than no-choice feeding tests²¹. Another important problems concerning practical application of antifeedants is habituation to them. Possibility of development of habituation against antifeedants can be confirmed from long term assays. Present study clearly revealed that 5% CA remained effective as an antifeedant for long period of time. As animals were not habituated to 5% CA treated bait for long duration, it can be effectively used as an antifeedant under field conditions to prevent the damage caused by house rats under commensal situations.

Tactile and feeding behavior of house rat in response to 5% CA in feed scale consumption monitor

The feeding behaviour of *R. rattus* (female n=3, male n=3) was recorded using feed scale consumption monitor in response to 5% CA treated bait. Quantitative data on number of feeding bouts, feeding bout time (s) and feed consumed (g) revealed that there was a significant reduction ($P \leq 0.05$) in the values of all the three parameters during treatment period as compared to pre-treatment period (Table 1). Rats not only reduced the consumption of treated bait but there was also a decline in the mean number of feeding bouts and mean length of feeding time during the treatment period indicating rats also handled the treated bait less frequently. Previous studies also reported reduction in consumption, feeding bouts events and feeding bout time in house rats exposed to bait treated with 0.4% cinnamamide and 5% CA^{15,22}.

Observation of rat behaviour in response to 5% CA revealed that rats closed their eyes when released in close chamber provided with treated bait. They also scratched their hands after taking each bout of treated bait. Reduction in feeding bout time and feeding events was also due to irritation caused by treated bait to the eyes and skin of rats forcing them to handle treated bait less frequently. As 5% CA treated bait caused irritation/inflammation to skin, rats preferred

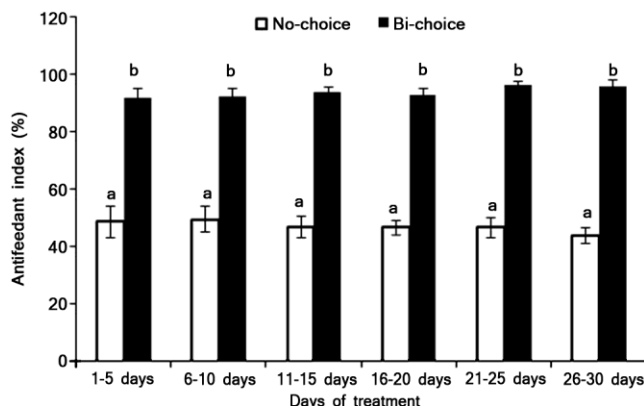


Fig. 1 — Comparison of antifeedant index (%) between no-choice and bi-choice feeding tests in rats. [a,b denotes significant difference in antifeedant index between no-choice and bi-choice feeding. 127×95 mm (1200×1200 DPI)]

Table 1 — Effect of 5% cinnamic aldehyde treated bait on feeding behaviour of *Rattus rattus* in feed scale consumption monitor

Gender	Days	Control			Treatment		
		No. of feeding bouts/h	Feeding bout time (s)/h	Consumption (g/100 g of bw) of untreated bait (g)/h	No. of feeding bouts/h	Feeding bout time (s)/h	Consumption (g/100 g of bw) of untreated bait (g)/h
Male	Day 1	9.16±0.13	1116±261.76	1.48±0.21	3.33±1.16	210.33±82.33	0.34±0.12
	Day 2	3.66±0.27	664.66±50.37	1.75±0.40	3±2.05	119.83±91.29	0.15±0.11
	Day 3	2±0.40	348±32.28	0.89±0.14	1.16±0.42	192±148.38	0.36±0.28
	Day 4	2.16±0.59	180.16±108.66	0.67±0.30	4.5±2.25	372±176.64	0.37±0.26
	Day 5	4.83±0.49	813.66±27.31	2.09±0.40	2.16±0.18	323.66±92.64	0.59±0.15
Female	Day 1	4.5±2.25	744±353.28	1.45±0.95	1±0.40	119.5±93.19	0.25±0.19
	Day 2	4.5±0	624.5±57.09	2.63±0.58	1.16±0.54	192±148.38	0.35±0.27
	Day 3	4.5±0.23	538.83±110.84	1.43±0.44	0.83±0.68	87±71.11	0.16±0.13
	Day 4	6±1.84	676.16±63.73	1.33±0.07	3.33±1.16	210.33±82.33	0.31±0.10
	Day 5	4.66±0.13	387.16±115.75	1.20±0.38	2.16±1.38	107.66±64.35	0.14±0.10
	Mean±SE	4.60±0.60 [#]	609.31±79.50 ¹	1.49±0.16*	2.26±0.37 ^{##}	193.43±28.12 ²	0.30±0.04**

[Values are Mean±SE, n=3 (each sex). [#], ^{##} denotes significant difference in feeding bout events/h between control and treated rats at 5% level of significance; ^{1,2} denotes significant difference in feeding bout time (s)/h between control and treated rats at 5% level of significance; and *, ** denotes significant difference in consumption (g/100 g body wt.) of bait between control and treated rats]

to eat alternative food under bi-choice conditions leading to more antifeedant effect in bi-choice feeding test (Fig. 1). The contact, fumigant and antifeedant effects of cinnamaldehyde were also reported against larvae and adults of *T. castaneum* and *S. zeamais*¹².

Effect of 5% CA on gastrointestinal tract of house rats

Colour of both stomach and intestine was changed to pinkish white/ black and their weight also reduced significantly in no-choice group in comparison to untreated and bi-choice groups of rats.

Histomorphometric and histomorphological studies of stomach

Anterior portion of stomach contains only non-glandular region and middle portion of stomach contains both glandular and non-glandular regions while posterior portion contains only glandular region. In between glandular and non-glandular regions, limiting ridge was seen in middle portion of stomach (Fig. 2A). Earlier studies reported that on the distal side of the limiting ridge, the stratified squamous epithelium of the non-glandular stomach changed abruptly to the simple columnar epithelium of the glandular region²³. Both non-glandular and glandular regions of stomach consist of 4 layers namely mucosa, submucosa, muscularis externa and serosa layer.

In no-choice group of rats, the thickness of keratin layer, glandular mucosa, submucosa and muscularis externa reduced significantly ($P \leq 0.05$) and the thickness of non-glandular mucosa was significantly increased ($P \leq 0.05$) as compared to untreated and

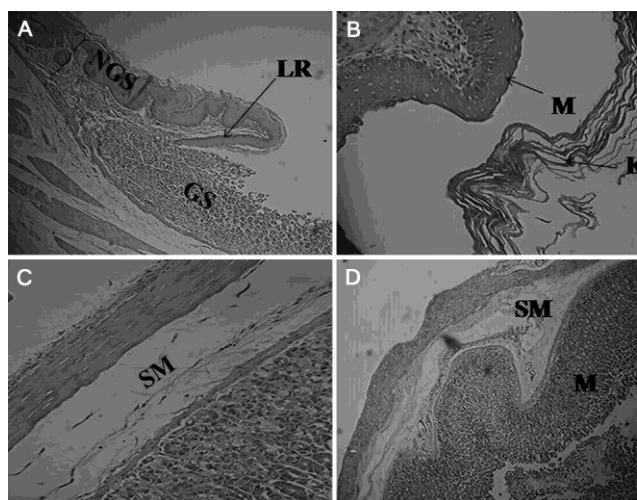


Fig. 2 — Showing (A) limiting ridge between glandular and non-glandular stomach; (B) Separation of keratin layer from mucosa of non-glandular stomach in no-choice group; (C) Removal of connective tissue, blood vessels and lymph vessels from sub-mucosa of no-choice group; and (D) Sub-mucosal invasion in mucosa of glandular region of stomach in no-choice group. NGS: Non-glandular stomach; GS: Glandular stomach; LR: Limiting ridge; KL: Keratin layer; SM: Sub-mucosa; M: Mucosa. Magnification 400X. 254×190 mm (600×600 DPI).]

bi-choice groups (Table 2). However, there was a non-significant difference in thickness of different layers of stomach between sexes and among different parts of the stomach except for thickness of mucosa. Increase in thickness of mucosal layer of non-glandular stomach both in no-choice and bi-choice groups of rats was due to hyperplasia of stratified squamous epithelium of non-glandular stomach and

Table 2 — Effect of 5% cinnamic aldehyde on thickness of different layers of stomach

Parts of stomach	Thickness of keratin (μm)		
	Untreated group	No-choice group	Bi-choice group
Anterior (non-glandular)	80.41 $\pm$ 8.72 ^b	25.83 $\pm$ 4.80 ^a	84.99 $\pm$ 7.17 ^b
Middle (non-glandular)	74.16 $\pm$ 4.89 ^b	36.24 $\pm$ 2.13 ^a	79.58 $\pm$ 2.46 ^b
	Thickness of mucosa (μm)		
Anterior (non-glandular)*	195.60 $\pm$ 9.22 ^c	396.22 $\pm$ 18.84 ^a	301.60 $\pm$ 14.56 ^b
Middle (non-glandular)*	185.71 $\pm$ 11.33 ^c	395.94 $\pm$ 19.03 ^a	331.55 $\pm$ 21.2 ^b
Middle (glandular)**	641.33 $\pm$ 31.22 ^c	454.33 $\pm$ 17.63 ^a	570.33 $\pm$ 15.21 ^b
Posterior (glandular)**	620.99 $\pm$ 19.76 ^c	401.16 $\pm$ 14.56 ^a	572.16 $\pm$ 7.53 ^b
	Thickness of submucosa (μm)		
Anterior	314.71 $\pm$ 12.34 ^b	188.60 $\pm$ 5.35 ^a	314.88 $\pm$ 5.94 ^b
Middle	315.38 $\pm$ 11.77 ^b	199.10 $\pm$ 9.79 ^a	296.27 $\pm$ 8.88 ^b
Posterior	311.88 $\pm$ 17.65 ^b	206.44 $\pm$ 15.98 ^a	276.78 $\pm$ 24.95 ^b
	Thickness of muscularis externa (μm)		
Anterior	323.11 $\pm$ 7.77 ^b	210.55 $\pm$ 8.23 ^a	309.6 $\pm$ 3.59 ^b
Middle	318.66 $\pm$ 6.29 ^b	200.16 $\pm$ 7.27 ^a	304.27 $\pm$ 14.00 ^b
Posterior	321.21 $\pm$ 20.42 ^b	209.10 $\pm$ 8.10 ^a	266.21 $\pm$ 21.24 ^b

[Values are mean $\pm$ SE; n=3 (each sex); Magnification-keratin layer: 400X, Mucosa, submucosa and muscularis externa: 100X. ^a, ^b and ^c denotes significant difference among different groups of rats at 5% level of significance; and *, ** denotes significant difference between glandular and non-glandular parts of stomach at 5% level of significance]

reduction in thickness of glandular stomach was due to degeneration of gastric glands present in mucosa of glandular stomach after treatment of rats with 5% CA. Even in bi-choice groups of rats, feeding insignificant quantity of 5% CA affected mucosa of both glandular and non-glandular parts of stomach. Previous study also reported hyperplasia of forestomach mucosa of rats treated with CA²⁴. Thickness of mucosa of glandular stomach was significantly more than that of non-glandular stomach due to the presence of gastric glands in glandular part of stomach. Earlier studies also reported that the mucosa of glandular region is thicker than the non-glandular region¹⁹. Keratin layer was also separated from the underlying tissue in no-choice group of rats (Fig. 2B). Function of keratin layer is to provide protection to underlying tissues of non-glandular stomach. Thinning and separation of this layer from underlying tissue reduced protection to non-glandular part of stomach. Connective tissue, blood vessels and lymph vessels were also removed from sub-mucosa of no-choice group (Fig. 2C). Consumption of 5% CA treated bait also caused number of other pathological changes in stomach of no-choice group like submucosal invasions in mucosa (Fig. 2D), focal hyperplasia with keratin and mucosal cysts in lumen, mucosa and submucosa in no-choice group of rats (Fig. 3 A-C). The muscularis layer is responsible for contraction and peristaltic movement in GI tract. Reduction in thickness of this layer in no-choice group might also reduce the peristaltic

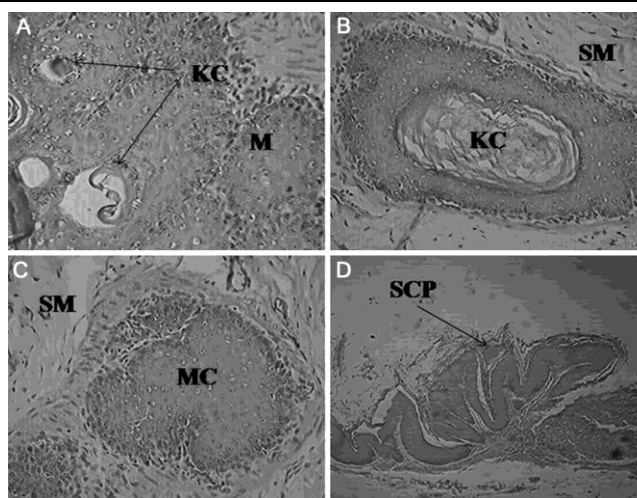


Fig. 3 — Showing (A-C) focal hyperplasia with keratin and mucosal cyst in mucosa and sub-mucosa; and (D) squamous cell papilloma in non-glandular region of stomach in no-choice group; [M: Mucosa; SM: Sub-mucosa; KC: Keratin cyst; MC: Mucosal cyst; SCP: Squamous cell papilloma. Magnification: 400X. 254 $\times$ 190 mm (600 $\times$ 600 DPI)]

movement, and thus movement and churning of food in stomach (Table 2). Animesh *et al.*²⁵ have reported significant decrease in the musculature with significant disintegration of tissues of submucosa and mucosa characterized by the presence of irregular masses of tissues with spaces between them in stomach of treated mice. There was, however, not much effect of 5% CA on thickness of submucosa, muscularis externa and keratin layer in bi-choice

group, due to the less or negligible consumption of treated bait by rats of this group (Table 2).

Gastric glands are present in mucosa of glandular stomach. These glands consist of mucosal, chief, parietal and neuroendocrine cells. Non-glandular mucosa consists of stratified squamous epithelium. Number of mucosal, parietal, chief and neuroendocrine cells decreased significantly in no-choice group of rats as compared with untreated and bi-choice groups of rats. There was also a significant difference in number of parietal cells/mm² between middle and posterior parts of stomach. Results also revealed that the number of stratified squamous epithelial cells were significantly more in no-choice group of rats followed by bi-choice and untreated group of rats indicating hyperplasia of these cells after treatment with 5% CA both in no-choice and bi-choice groups of rats although affect of 5% CA was mild in bi-choice group of rats because of consumption of very small quantity of treated bait (Table 3). Squamous cell papilloma was also seen in non-glandular region of stomach in no-choice group of rats (Fig. 3D). Squamous cell papilloma in non-glandular stomach was also reported earlier²⁶. Papillomas are localized proliferations of the non-glandular stomach mucosa. Empty spaces were observed in place of glands in mucosa of glandular stomach due to degeneration of gastric glands and

thus reduction in number of different cells lining the gastric glands (Fig. 4 A and B). Gastric lesions and cellular degeneration of gastric region were earlier reported in rats fed on bait containing 1 and 2% red chilli powder²⁷.

Histomorphometric and histomorphological studies of small intestine

There was a significant ($P \leq 0.05$) difference in tubule diameter, lumen diameter and length of villi among duodenum, jejunum and ileum. The tubule diameter, lumen diameter and length of villi were significantly more in duodenum as compared to that of jejunum and ileum. Villi length was more in duodenum as compared to jejunum and ileum¹⁸. There was, however, a non-significant difference in tubular diameter among different groups but lumen diameter was significantly more in duodenum in no-choice group of rats as compared to that in untreated and bi-choice group of rats. The mean villus length in no-choice group of rats was found to be significantly ($P \leq 0.05$) reduced as compared to bi-choice and untreated groups of rats (Table 4). Reduction in the length of villi and thickness of mucosa, submucosa and muscularis externa in no-choice group resulted in increase in lumen diameter.

The mean thickness of mucosa, sub-mucosa and muscularis externa was also significantly less ($P \leq 0.05$) in no-choice group of rats as compared to

Table 3 — Effect of 5% cinnamic aldehyde on number of cells/mm² of glandular and non- glandular stomach mucosa

Parts of stomach	No. of mucosal cells/mm ²		
	Untreated group	No-choice group	Bi-choice group
Middle	3.56±0.18 ^b	2.56±0.12 ^a	3.61±0.18 ^b
Posterior	3.73±0.20 ^b	2.64±0.11 ^a	3.66±0.12 ^b
	No. of parietal cells/mm ²		
	Untreated group	No-choice group	Bi-choice group
Middle*	4.22±0.11 ^b	3.30±0.11 ^a	4.21±0.12 ^b
Posterior**	4.25±0.17 ^b	2.94±0.10 ^a	3.96±0.09 ^b
	Number of chief cells /mm ²		
	Untreated group	No-choice group	Bi-choice group
Middle	4.10±0.12 ^b	1.86±0.08 ^a	4.29±0.12 ^b
Posterior	4.13±0.14 ^b	1.95±0.06 ^a	4.17±0.10 ^b
	No. of neuroendocrine cells/mm ²		
	Untreated group	No-choice group	Bi-choice group
Middle	0.71±0.05 ^c	0.16±0.04 ^a	0.50±0.04 ^b
Posterior	0.68±0.05 ^c	0.15±0.04 ^a	0.63±0.03 ^b
	No. of cells of stratified squamous epithelium/mm ²		
	Untreated group	No-choice group	Bi-choice group
Ant. stomach	1.98±0.07 ^a	3.80±0.24 ^c	2.38±0.13 ^b
Mid. stomach	1.97±0.07 ^a	3.81±0.06 ^c	2.49±0.23 ^b

[Values are mean±SE; n=3 (each sex); Magnification: 400X. ^a and ^b denotes significant difference among different groups of rats at 5% level of significance; and *, ** denotes significant difference between different parts of stomach at 5% level of significance]

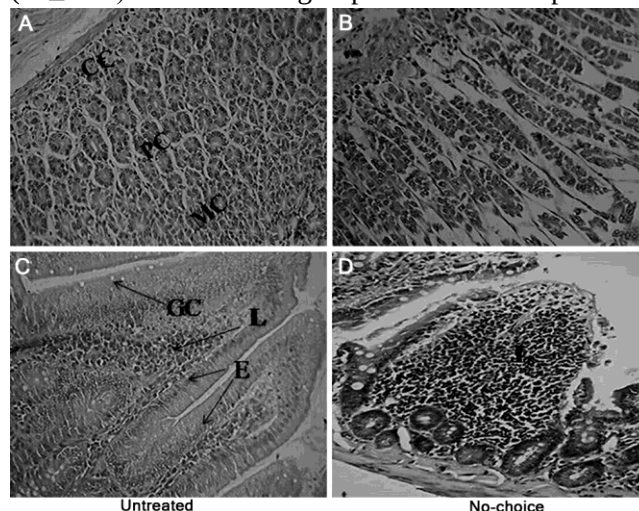


Fig. 4 — Showing (A & B) reduction in number of gastric glands, mucosal, chief and parietal cells in glandular region of stomach in no-choice group; and (C & D) aggregates of lymphocytes and reduction in number of enterocytes and goblet cells in villi of no-choice group as compared to untreated group. [MC: Mucosal cells; PC: Parietal cells; CC: Chief cells; GC: Goblet cells; L: lymphocytes; E: Enterocytes. Magnification: 400X. 254×190 mm (600×600 DPI)]

that of bi-choice and untreated groups of rats. There was no effect on intestine of bi-choice group which may be due to less or negligible consumption of treated bait by rats. There was, however, a non-significant difference in thickness of different layers among duodenum, jejunum and ileum (Table 5). In no-choice group of rats, loose connective tissue was degenerated from the sub-mucosa. Earlier studies reported atrophy of musculature and areolar connective tissue of the submucosa in treated mice²⁵.

Table 4 — Effect of 5% cinnamic aldehyde on diameter and villi length of small intestine

Part of small intestine	Tubular diameter (mm)		
	Untreated group	No-choice group	Bi-choice group
Duodenum *	2.82±0.13	2.75±0.04	2.81±0.09
Jejunum **	2.14±0.03	2.08±0.03	2.19±0.04
Ileum ***	1.99±0.06	1.96±0.04	1.98±0.07
Lumen diameter (mm)			
Duodenum*	1.92±0.13 ^a	2.24±0.06 ^b	1.96±0.10 ^a
Jejunum **	1.59±0.03 ^a	1.62±0.03 ^a	1.53±0.03 ^a
Ileum ***	1.43±0.06 ^a	1.51±0.05 ^a	1.43±0.07 ^a
Villi length (µm)			
Duodenum***	399.88±7.11 ^c	229.71±6.78 ^a	385.56±10.83 ^b
Jejunum**	369.39±2.50 ^b	223.01±9.41 ^a	365.12±13.24 ^b
Ileum*	341.37±4.37 ^b	213.46±12.95 ^a	347.69±7.05 ^c

[Values are mean±SE; n=3 (each sex); Magnification: 400X. *, **, *** denotes significant difference among different parts of small intestine at 5% level of significance; ^a, ^b and ^c denotes significant difference among different groups of rats at 5% level of significance]

Table 5 — Effect of 5% cinnamic aldehyde on thickness of different layers of small intestine

Parts of small intestine	Thickness of mucosa (µm)		
	Untreated group	No-choice group	Bi-choice group
Duodenum	298.60±9.44 ^b	197.77±4.63 ^a	277.21±6.86 ^b
Jejunum	299.99±5.30 ^b	197.50±5.20 ^a	273.27±12.65 ^b
Ileum	297.49±5.44 ^b	175.183±15.10 ^a	270.27±7.36 ^b
Thickness of sub-mucosa (µm)			
Duodenum	54.44±3.12 ^b	32.01±2.12 ^a	53.74±2.63 ^b
Jejunum	56.38±1.93 ^b	29.02±2.61 ^a	54.06±2.08 ^b
Ileum	52.01±1.90 ^b	31.87±1.58 ^a	55.65±1.70 ^b
Thickness of muscularis externa (µm)			
Duodenum	55.07±1.93 ^b	29.09±0.70 ^a	54.65±2.49 ^b
Jejunum	52.77±0.94 ^b	26.73±1.18 ^a	53.55±3.25 ^b
Ileum	53.95±2.13 ^b	29.44±1.41 ^a	55.79±3.66 ^b

[Values are mean±SE; n=3 (each sex); Magnification: 400X. ^a, ^b and ^c denotes significant difference among different groups of rats at 5% level of significance]

Enterocytes are tall columnar cells with surface microvilli and are present in surface epithelium of villi. Goblet cells are scattered among the enterocytes and are present in the surface epithelium lining the villi. In no-choice group of rats, the mean number of lymphocytes was significantly ($P \leq 0.05$) increased and the number of enterocytes and goblet cells were significantly ($P \leq 0.05$) decreased as compared to that of bi-choice and untreated groups (Table 6; Figs 4 C & D). Cytoplasmic disruption has been reported to result in reduced number of absorptive, goblet, entero-endocrine and paneth cells of columnar epithelium which lead to formation of numerous vacuoles²⁵. Reduction in the number of enterocytes after CA treatment in no-choice group of rats might have resulted in decreased ion uptake, water uptake, sugar uptake, peptide and amino acid uptake, lipid uptake, vitamin B₁₂ uptake, resorption of conjugated bile salts and secretion of immunoglobulins and in absorption of various other nutrients from the digestive tract. However, increase in number of lymphocytes in villi of small intestine indicates the inflammation of small intestine in no-choice group of rats. Aggregates of lymphocytes and their infiltration in lamina propria were also recorded in no-choice group of rats (Figs 4 C & D). Lymphocyte infiltration in lamina propria was also reported in rats fed on 1 and 2% red chilli powder²⁷. Incorporation of essential oils in food was reported to cause hypertrophy of mucosal membrane of intestinal villi and leukocytes infiltration²⁸. High dose of aqueous garlic extract caused vacuolations,

Table 6 — Effect of 5% cinnamic aldehyde on number of cells of villi

Parts of intestine	Number of enterocyte cells /mm ²		
	Untreated group ^b	No-choice group ^a	Bi-choice group ^b
Duodenum	3.51±0.09	1.91±0.12	3.64±0.17
Jejunum	3.99±0.13	1.85±0.16	3.85±0.18
Ileum	3.70±0.06	1.73±0.10	3.41±0.24
No. of lymphocytes/mm ²			
Duodenum	2.45±0.09	3.87±0.19	2.44±0.03
Jejunum	2.52±0.09	3.81±0.13	2.41±0.11
Ileum	2.36±0.14	3.64±0.20	2.37±0.11
No. of goblet cells/mm ²			
Duodenum*	0.49±0.05	0.15±0.02	0.46±0.04
Jejunum**	0.57±0.06	0.21±0.02	0.59±0.04
Ileum***	0.62±0.05	0.13±0.02	0.59±0.05

[Values are mean±SE; n=3 (each sex); Magnification: 400X. *, **, *** denotes significant difference among different parts of intestine at 5% level of significance; ^a and ^b denotes significant difference among different groups of rats at 5% level of significance]

cell death, loss of epithelial cells and degeneration of the brush borders and reduction in the number of goblet cells²⁹.

Crypts of lieberkuhn are present in lamina propria of mucosa. Results revealed that there was a significant ($P \leq 0.05$) difference in the number of crypts among untreated, no-choice and bi-choice group of rats with least number in no-choice group of rats (Table 7). Number of stem cells increased significantly ($P \leq 0.05$) in no-choice group as compared to that in untreated and bi-choice group of rats. However, the mean number of paneth and neuroendocrine cells was significantly less ($P \leq 0.05$) in no-choice group as compared to bi-choice and untreated groups (Table 7). Hence, it is clear from the results that the number of different cells of intestinal mucosa reduced significantly in no-choice group of rats except stem cells and lymphocytes as their number increased. Empty spaces were observed in mucosa of intestine in no-choice group of rats because of degeneration of intestinal glands (Fig. 5) resulting in reduction in number of different cells lining the intestinal glands/crypts of lieberkuhn. Reduction in number of different cells of intestinal glands might have caused reduction in secretion of intestinal juice and hormones secreted by neuroendocrine cells of intestinal glands causing indigestion in rats, and thus reduced consumption of bait in no-choice group

of rats. Number of stem cells of intestinal glands increased to compensate the degeneration of different cells of intestinal glands (Table 7).

In no-choice group, forceful feeding of treated bait resulted in histopathological changes in gastrointestinal tract leading to significant reduction in consumption of treated bait. But in bi-choice feeding test, GI tract was not affected (Fig. 6) as rats avoided treated bait in the presence of alternative plain bait. So reduction in consumption of treated bait in bi-choice group of rats might be due to primary repellent effect of 5% CA treated bait through olfaction or through inflammation of skin of rats as was clear from the changes in tactile behaviour of rats in response to 5% CA.

Repellents can be classified as either primary or secondary, according to their site of activity in the target species³⁰. Primary repellents provoke instantaneous responses through taste, olfaction, or irritation of the buccal cavity. Secondary repellents produce distressing effects after eating on gastrointestinal tract. Some repellent compounds have both primary and secondary repellent effects³¹. Results from the present studies also indicate that 5% CA is not only acting as secondary repellent/ antifeedant against house rats affecting

Table 7 — Effect of 5% cinnamic aldehyde on number of crypts of lieberkuhn and cells/mm² of intestinal mucosa

Part of intestine	Number of crypts of lieberkuhn /mm ²		
	Untreated group	No-choice group	Bi-choice group
Duodenum	0.37±0.06 ^c	0.09±0.01 ^a	0.29±0.01 ^b
Jejunum	0.32±0.02 ^c	0.08±0.01 ^a	0.28±0.02 ^b
Ileum	0.30±0.01 ^c	0.07±0.007 ^a	0.30±0.01 ^b
No. of stem cells/mm ²			
Duodenum	0.11±0.02 ^a	0.66±0.05 ^b	0.09±0.01 ^a
Jejunum	0.13±0.02 ^a	0.68±0.03 ^b	0.14±0.02 ^a
Ileum	0.10±0.02 ^a	0.70±0.03 ^b	0.09±0.02 ^a
No. of paneth cells/μm			
Duodenum	7.68±0.48 ^b	3.07±0.24 ^a	7.17±0.51 ^b
Jejunum	7.68±0.51 ^b	3.07±0.65 ^a	8.02±0.61 ^b
Ileum	7.85±0.61 ^b	3.34±0.56 ^a	7.85±0.38 ^b
No. of neuroendocrine cells/μm			
Duodenum	6.01±0.14 ^b	1.42±0.27 ^a	5.84±0.27 ^b
Jejunum	5.85±0.37 ^b	1.21±0.13 ^a	5.86±0.26 ^b
Ileum	5.52±0.14 ^b	1.94±0.31 ^a	5.68±0.27 ^b

[Values are mean±SE; n=3 (each sex); Magnification: 400X. ^a and ^b denotes significant difference among different groups of rats at 5% level of significance]

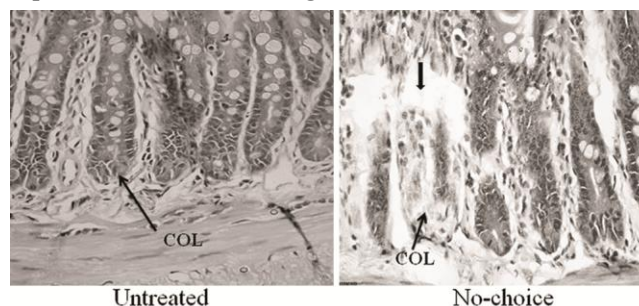


Fig. 5 — Shows empty spaces because of degeneration of Crypts of Lieberkuhn (COL) in no-choice group of rats

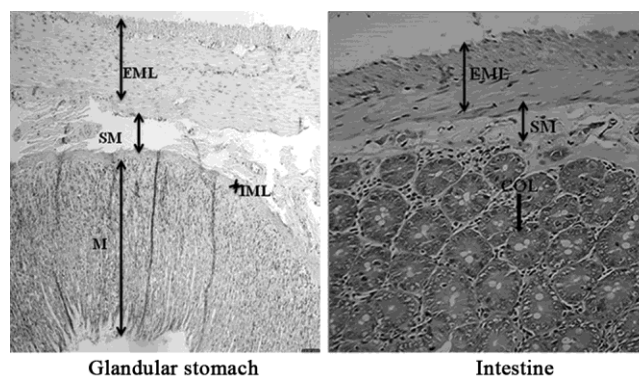


Fig. 6 — Shows no effect of treatment on glandular stomach and intestine in bi-choice group of rats. External muscularis layer (EML), Submucosa (SM), Mucosa (M), Internal muscularis layer (IML), Crypts of Lieberkuhn (COL)

gastrointestinal tract of rats, but might also be causing irritation to skin leading to development of primary repellent effect also although this effect might not develop immediately after exposure of rats to 5% CA treated bait. Earlier study in our laboratory reported that repellent effect of 5% CA develops sometime between 4-24 h of exposure of rats to 5% CA¹⁵. A recent study also revealed repellent effect of eucalyptus oil against house rat⁹.

It is concluded from the present investigation that 5% cinnamic aldehyde (CA) affects both the gastrointestinal tract and the skin of rats indicating both primary and secondary repellent effect of 5% CA treated bait.

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