

Gastric damage due to Deltamethrin at maximum residue level (MRL) and its auto-reversal in adult Wistar rats

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ABSTRACT

Introduction: Deltamethrin is a useful pesticide when applied judiciously to crops. However, it has also dose-dependent toxic effects on stomach once ingested with sprayed crops. Its maximum permissible limit (MRL, Maximum Residue Level) as pesticide food residues is 5mg/kg body weight as defined by WHO. Though there is still a potential of causing harm at these levels and may be a serious potential health hazard.

Objective: To determine the severity of gastritis induced by Deltamethrin administered at MRL doses to adult Wistar rats and the extent of spontaneous auto-reversal over three weeks.

Materials & Methods: In this two-phase experimental subacute toxicity study of seven weeks carried out at the Institute of Basic Medical Sciences, Khyber Medical University, Peshawar, Pakistan, 24 adult Wistar rats were divided into six subgroups. In Phase I, two negative control sub-groups (A-1, A-2) were kept on normal diet while two positive control sub-groups (B-1, B-2) were given oral Indomethacin (2mg/kg body weight) to induce gastritis. One of each control groups was then dissected, while other one left under observation for auto-reversal in phase II. Out of the two treatment sub-groups (C1, C-2), one group was given deltamethrin (5mg/kg body weight) orally in phase I and then culled. The second group was first given deltamethrin (5mg/kg body weight) in phase I and then left under observation for auto-reversal, during phase II and all were culled at its end. The stomach samples were collected and processed to obtain histology slides for analysis via microscopy and micrometry. Grading was done to look for changes according to the Visual Analogue Scale (VAS), and Operative Link on Gastritis Assessment (OLGA).

Results: The experimental Deltamethrin group when compared to negative and positive control groups, revealed moderate changes in rat stomach histomorphology while auto-reversal group showed mild severity changes.

Conclusion: Deltamethrin administered in a permissible MRL dose induced substantive changes in rat gastric mucosa, with partial spontaneous auto-reversal of gastritis, showing the potential persistent danger of Deltamethrin once it has been introduced into the body.

Keywords: Pyrethrins; Gastritis; Visual Analogue Scale (VAS); Rats, Wistar.

The authors declared no conflict of interest. All authors contributed substantially to the planning of research, data collection, data analysis, and write-up of the article, and agreed to be accountable for all aspects of the work.

INTRODUCTION

Pesticides are beneficial in view of the exploding global population because they increase the crop's nutritional value and outcome by decreasing the load of pests and diseases.¹ A pyrethroid is a man-made insecticidal compound similar to the natural pyrethrin which is produced by the flowers *Chrysanthemum cinerariaefolium* and *C. coccineum*.^{2,3} Though other pyrethroids are made up of more than one compound, Deltamethrin consists of only one pure compound and makes up 85% of total production.⁴ Deltamethrin is also used to protect stored food items such as cereals, grains, and coffee beans.³ Other uses include insect control for public health concerns, pest control in forestry, pest control in animal facilities, parasite control in animals, and as a wood preservative.⁵

Due to extensive use of Deltamethrin on our food and the ingestion of its residues by humans it may cause a variety of toxic symptoms from acute to chronic depending on the amount taken in. Each food has a certain permissible level of pesticides residue determined by World Health Organization (WHO) with a range of 2-5mg/kg for edible foods.⁶⁻⁸

The question arises as to the effects of these residue pesticides present in food on our biological systems. In this modern era some of the conditions like heartburn or gastritis are increasing in severity and frequency due to factors like lifestyle, smoking, alcohol intake and *H. pylori* infestation. But the risk related to residue pesticide contaminated food cannot be ignored, as it may be an important underlying cause.⁹

The present study was carried out to identify the histomorphological changes in gastric mucosa of laboratory rats with reference to Maximum Residue Level (MRL) of Deltamethrin and to identify auto-reversal of the changes within a given time period after stoppage of Deltamethrin.

MATERIALS & METHODS

This experimental, laboratory-based study was carried out at the Institute of Basic Medical Sciences (IBMS), Khyber Medical University (KMU), Peshawar, Khyber Pakhtunkhwa, Pakistan

from August 2016 to January 2018 after approval from the Advanced Studies and Review Board (ASRB) and University Ethical Board.

The current results are part of a bigger ongoing study to be published subsequently.

The ARRIVE guidelines and FRAME protocol for animal use in laboratories were followed. Twenty four adult Wistar rats were acclimatized and then divided into three main groups, A and B (control groups) and C (experimental group), as shown in Table 1.

The experimental period was four weeks for phase I and three weeks for phase II. Details of grouping and dosages of chemicals used are shown in table 1. At the end of Phase I, animals in Group A1, B1 and C1 were culled, and their stomachs were processed for microscopic analysis.

After culling using chloroform, the abdominal cavity was opened ventrally, and stomach identified and separated by clamping at cardiac and pyloric regions. It was then opened along the greater curvature and washed with tap water. The glandular part of stomach was identified and separated from rest of stomach. After washing and separating the glandular part it was preserved in 10% buffered formalin solution. After completing the dissection, the carcass was disposed according to Use of Animals in Laboratory science guidelines. The preserved samples were then processed and stained with haematoxylin and eosin (H&E) and periodic acid Schiff (PAS).

Nikon multi-head microscope with a display and camera was used for examination and imaging of slides at three magnifications i.e., 40x, 200x, 400x. and final observations were entered into the following scoring systems divided into two levels.

Table 1: Details of grouping and dosages of chemicals administered (n=24).

Groups	No. of Rats	Sub-groups		Follow up Time Period	
				Phase I	Phase II
				28 Days	21 Days
Group-A (negative Control)	8	A-1	4	Normal Diet + Vehicle (Sesame oil)	-
		A-2	4	Normal Diet + Vehicle (Sesame oil)	Normal Diet + Vehicle (Sesame oil)
Group-B (positive Control)	8	B-1	4	Indomethacin (2 mg/kg/day)	-
		B-2	4	Indomethacin (2 mg/kg/day)	No drug given (kept under observation for auto-reversal)
Group-C (experimental)	8	C-1	4	Deltamethrin (5 mg/kg/day)	-
		C-2	4	Deltamethrin (5 mg/kg/day)	No drug given (kept under observation for auto-reversal)

The Visual Analogue Scale (VAS) was applied in which inflammatory infiltrates were categorized into mild, moderate, and severe. According to this scale, maximum of five lymphocytes, plasma cells and macrophages per high-power (x40 objective) is considered "Normal" ⁹⁻¹² with no neutrophils ¹³. Mild has neutrophils in mucosa, in moderate infiltrates extend to muscularis mucosae with glandular hypoplasia and the severe grade has monocyte infiltration as well as glandular atrophy.

Next the extent of glandular atrophy was observed and classified as mild, moderate, and severe. This classification is according to the international system for gastritis scoring Operative link on gastritis assessment (OLGA)^{13,14}. In this system the percentage of atrophy is observed at compartment level (antrum including incisura angularis and corpus) and then combining all the compartments to get the total OLGA score.

Statistical analysis was performed using the (SPSS) version 20. Statistical results are given as mean and standard deviation for continuous variables. Frequencies are calculated for categorical variables. Paired sample t test is used to examine mean of two groups (treated and non-treated). ANOVA test was applied to compare the means of all the groups included in study, a p- value of ≤ 0.05 is considered as statistically significant. Significance between the groups was provided by Post Hoc (Tukey HSD).

RESULTS

Deltamethrin in a dose of 5mg/kg was given via oral gavage along with food and water in the experimental groups (C1 and C2). These were compared with the negative and positive control groups (A and B) and results analyzed according to the visual analogue scale and OLGA scoring. Scoring was based on the average scores of corpus and antrum of each group.

All the rats of Group C that received Deltamethrin showed decrease in weight over the period of 28 days (p value<0.04, Post-hoc Tukey).

Visual Analogue Scale (VAS)

According to VAS there were occasional inflammatory cells (no neutrophil and <5 lymphocytes) present in the mucosa particularly in the lamina propria and between the glands which were considered as "Normal" in the control Group A (Figure 1, A).

Indomethacin Group (B) showed moderate number of inflammatory cells, both lymphocytes and neutrophils, in the lamina propria of oxyntic and antral parts.

Deltamethrin group (C1) showed mild to moderate number of neutrophils, lymphocytes, and decreased number of monocytes (Figure 1).

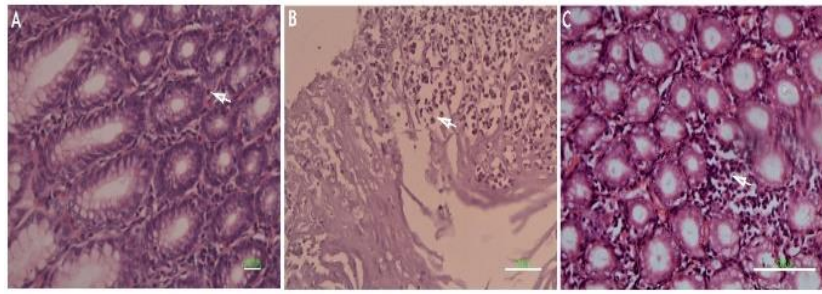


Figure 1- Cross section of corpus illustrating the visual analogue scale (VAS).

A- VAS Normal. B1- VAS Moderate to Severe, C1- VAS Mild to Moderate. White arrows indicating the inflammatory cells in lamina propria. Scale 200µm.

Visual analogue scale in Antrum

Mild to moderate inflammatory cells were present in antrum region of stomach in Deltamethrin treated group (C1). However,

Indomethacin (B1, positive control) group showed moderate to severe inflammatory cells (Figure 2).

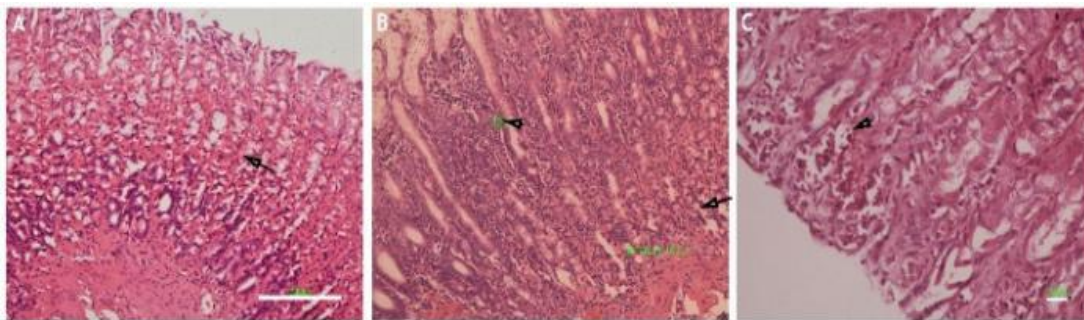


Figure 2: Longitudinal section of Antrum showing normal inflammatory cells in Group A. Moderate to severe cells (Black arrow) in Indomethacin group (B1). However mild inflammatory cells (Black arrow) are present in the antrum region of Deltamethrin treated group (C1).

OLGA (Atrophy Scoring) in Deltamethrin treated group

There were mild atrophic changes in gastric mucosa after counting the glands and assessing the atrophy in Deltamethrin treated group (23.33±13.97). In positive control group indomethacin atrophy score was moderate (32.6±13.35). Post hoc test revealed significance in atrophy score when Deltamethrin group was compared with Normal control group “A” (*p* value <0.001). However, there was no significance between B1 and C1 (*p*= 0.74).

When analyzed by OLGA system Negative control group A showed grade 0. Positive control group showed grade 2 score. And Deltamethrin group showed grade 1 atrophy score. Representative images (corpus and antrum) of the Deltamethrin group along with negative (A) and positive control (B1) group are given below (Figure 3).

Corpus

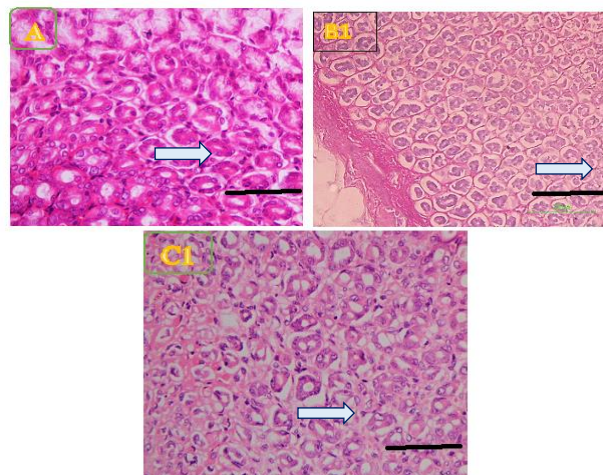


Figure 3: Cross section of Corpus- A- There is no atrophy in the negative control group. B1- Indomethacin treated group showed moderate atrophy in glands. C1- Deltamethrin treated group showing mild atrophy in gastric glands (H&E)

Auto-reversal Group

C2 were those rats who were kept in phase “2” and given only food for observing auto recovery after administering Deltamethrin in phase “1”. According to visual analogue scale and OLGA their results were interpreted as:

Visual analogue scale

Auto reversal Group (C2) showed decrease in inflammatory cells after stoppage of Deltamethrin. On other hand auto recovery group of Indomethacin also showed decrease in inflammatory cells. C/S of lamina propria is highlighted in a section of corpus in figures 3-8.

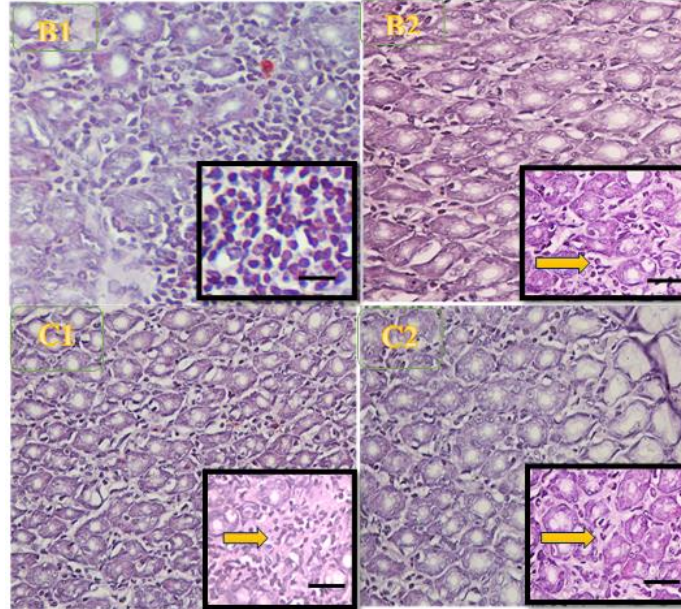


Figure 4: Cross section of Corpuses of Indomethacin auto reversal group (B2) and Deltamethrin auto reversal group (C2) showed decrease in inflammatory cells as compared to B1 and C1 respectively. According to visual analogue scale both B2 and C2 are categorized as mild inflammatory groups. (PAS, scale bar 2µm magnified area)

OLGA scoring for Auto-reversal Group

Deltamethrin auto recovery Group (C2) results showed significant reversal of atrophic changes (5.550 ± 7.33) with a p value <0.001 when compared with Deltamethrin group C1, Also C2 results were compared with the auto reversal group of positive

control group (Indomethacin treated in phase 1) B2 (8.00 ± 7.70) which revealed a non-significant p value >1 . Comparison between the groups are shown by the representative images of Corpus and antrum from each group in figures 5 and 6 respectively.

Corpus

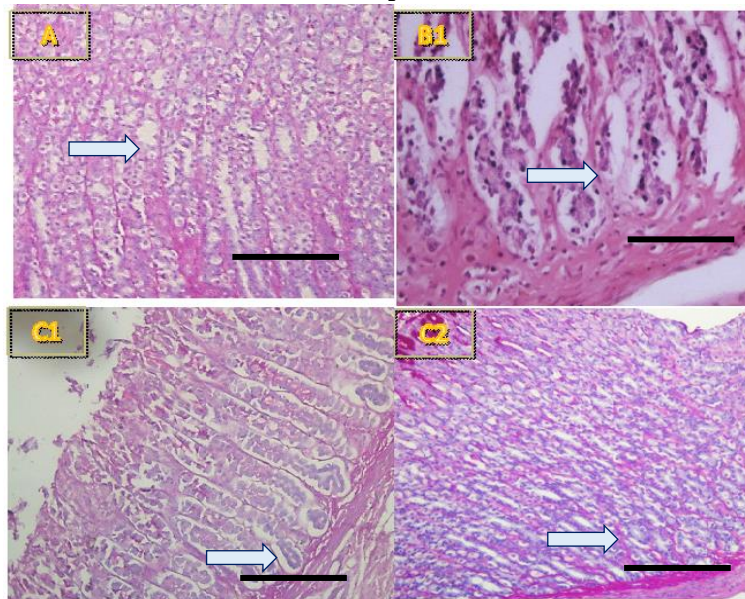


Figure 5: Longitudinal section of Corpus of A; Negative control group showed n atrophy. B1; Positive control group showed moderate atrophy. C1; Mild atrophy of gastric glands. C2; Auto reversal group of Deltamethrin showed normal morphology. (PAS).

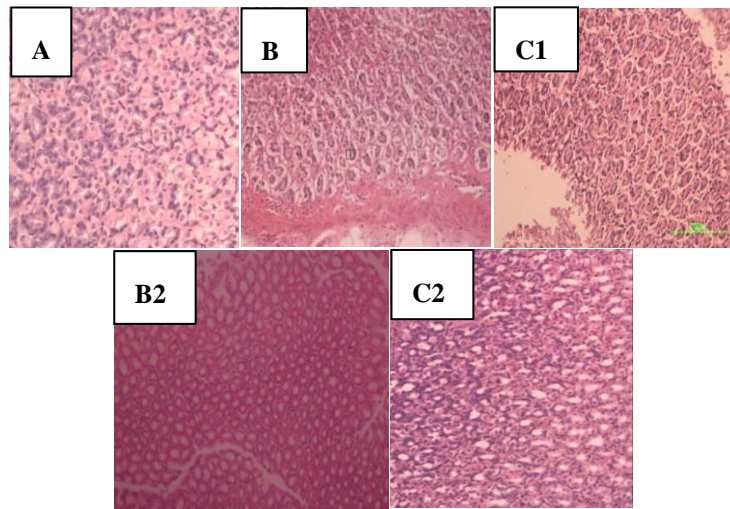


Figure 1: Cross section of Antral glands. Auto reversal group of rodents treated with Deltamethrin (C2) is compared with Group A, B1, B2 and C1. (H&E, scale bar 200µm)

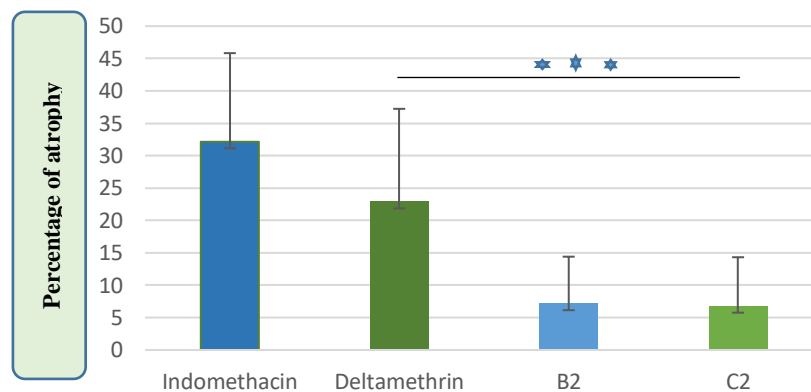


Figure 2: Bar chart showing significant reduction of atrophy in C2 when compared with Deltamethrin group ($P < 0.01$). Auto reversal group of indomethacin B2 also showed reduction of atrophy as compared to Indomethacin group (P value 0.04).

DISCUSSION

The primary aim of this study was to examine the effects of pesticide (Deltamethrin) in its residue form on our stomach mucosa ingested with food. Results of our study revealed that Deltamethrin when given orally even in residue quantity caused significant damage to the gastric tissue in phase I. The amount of damage was measured by visual analogue scale for inflammatory infiltrates by Dixon MF, et al¹⁰ and atrophy of glands was measured by OLGa score¹³. Cumulative result of Deltamethrin treated group was labeled as “mild atrophy”. It was compared with the results of indomethacin treated group which was labeled as “moderate atrophy”. When Deltamethrin was stopped in phase two significant recovery was noted in the morphology of stomach mucosa.

Reduction in weight: Our study showed a significant weight loss in Deltamethrin treated rats as has also been demonstrated in previous studies with Pyrethroids that reduce the weight even in residue quantity both under laboratory and field conditions.¹⁵ A study conducted on randomly selected non-targeted aquatic animals revealed that Deltamethrin along with other pesticides reduces the body weights of aquatic animals.¹⁶ They examined the water sediment samples of various pesticides. An article was published in 2016 on the effects of Deltamethrin on rats’ testes in

which effects of different doses of Deltamethrin on body weights were also mentioned.¹⁵ Three types of Deltamethrin doses were administered to three different groups of rodents. The results showed that there was no significant weight reduction by administering 1mg/kg and 5mg/kg per day. However weight reduction was observed in a dose of 10mg/kg/day. In contrast, our study results revealed reduction in body weights even by administering 5mg/kg/day.

Deltamethrin causes gastritis: This subacute toxicological study showed that in a dose of 5mg/kg per day Deltamethrin when given orally alone caused significant damage to the gastric tissue. Indomethacin showed a much more severe effect as revealed by previous studies and is notorious for its gastritis inducing capability.¹⁷⁻¹⁹ Deltamethrin group (C1) resulted in hemorrhage and congestion in the mucosal part on gross observation. Histologically there was mild to moderate inflammation and atrophy. On clinical studies in humans, toxicological studies have shown that even in MRLs, Pyrethroids can cause nausea, vomiting or diarrhea but with no specific histological data on gastritis.²⁰ A review mentioned that ingesting Deltamethrin can cause abdominal cramps, nausea and vomiting (symptoms of gastritis) along with a myriad of other neurological symptoms.²¹ Thus, keeping in view, all these studies we can

deduce that these pesticides even in minimal permissible levels in our routinely ingested food can have mild to moderate effects on our stomach and can cause significant damage to our gastric mucosa. Besides the effects of MRLs, there are some studies that were done on the effects of Pyrethroids related gastritis, particularly in Deltamethrin acute poisoning, where there is erosion of gastric mucosa leading to hematemesis.^{22,23}

Auto reversal study “Auto reversal” groups included two groups (B2 & C2) which showed decrease in inflammation as well as atrophy score as compared to B1 and C1. Like previous studies when causative agents were removed there was reversal of gastric inflammation and atrophy.²⁴ However, specific histological study regarding Deltamethrin auto reversal was not found.

LIMITATIONS

A sample size created a large standard deviation in statistical analysis. It was due to a smaller number of available albino rats

at that time. In spite of these we have identified significant changes and results but increasing the sample size would increase the robustness of the study.

CONCLUSION

Deltamethrin causes auto-reversible inflammatory and atrophic changes in gastric mucosa of adult Wistar rats even in Maximum Residue Level (MRL).

RECOMMENDATIONS

There should be minimal use of pesticides and natural farming should be promoted as it is the only way by which we can prevent our health from hazardous pesticides.

Further research should be done on molecular level, and long term studies should be conducted for the effects of Deltamethrin on human health.

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