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The Effect Of Pesticide Residues On The Small Intestinal Morphometry Of Rats.

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ABSTRACT

The study of the effect of pesticide residues on the small intestinal morphometry of rats was undertaken using thirty Albino (Wistar) rats, which were separated into three groups (A, B, and C). The first group (A) was the control group and was fed normal rat chow. The first test group (B) was fed with the experimental diet after seven weeks of storage with the pesticide while the second test group (C) was fed with the experimental diet after fourteen weeks of storage with the pesticide. The pesticide of choice for the study was the cooper storage grain powder mixed at the rate of 0.5kg per tone and the feeding period after the storage lasted fourteen weeks. The results revealed that small intestinal morphometry of animals in the test groups were affected when compared with the control. The mucosa and the villi were reduced in height. The surface amplification factor due to the jejunal villi was 7, 4 and 5 for groups A, B. and C. respectively of which the level of difference was significant between the control group (A) and the test groups (B and C) ($p<0.05$), and in-between the test groups (A and B) ($p<0.05$).

Key words: Pesticide Residues; Permethrin; Morphometry; Small Intestine;

Excessive loss of food crops and stored foods to insects and other destructive pests contributes to starvation, which is an obvious health problem in many parts of the world. Pesticides have been effectively used to repel and control unwanted agents that would drastically reduced food production (Newman, 1978; Iyaniwura, 1996). Pesticides are biologically active compounds extensively used to control pests like insects, weeds, fungi and rodents that endanger the production, transportation and storage of foods (Casarett & Doul, 1975; Seth, 1996). These pesticides are also used to eradicate organisms involved in the transmission of human, plant and animal diseases (Newman, 1978). Pesticides occupy a rather unique position among the many chemicals that man encounters daily, in that they are deliberately added to the environment or food crops for the purpose of killing or injuring some forms of life (Casarette and Doul, 1975; Timbrell, 1982).

A pesticide could be selective in toxicity, if it is highly toxic only to a few related organisms or broad spectrum in toxicity if it has a high toxicity to a wide range of organisms (Matsumura, 1975). However most of the chemicals used as pesticides are not highly selective but are generally toxic to many non-target species including man and animals that co-habit the environment (Metcalf, 1975; Seth, 1996).

Some of these pesticides remain as residues according to Kwanashie, (1990) due to the possibilities of non-uniformity of mixing, unwitting repetition of treatment and over dosing by unsupervised, ignorant and illiterate peasant farmers and traders. There is the danger of residual effect of treated products when brought to the market for consumption prior to the time allowed for he residual effects to reach tolerable level. This is observed in our market due to the urge to maximize profit at the detriment of the health of the consumers.

Since the small intestine is the site of most of the digestion and absorption in the body, any derangment of the small intestine may lead to malabsorption, which impairs growth and other body functions. It is in the light of the above, that Coopex® grain storage powder which contains permethrin as the active component was used to study the effect of pesticide residues on the morphometry of the small intestine of rats.

MATERIALS AND METHODS

The pesticide used for this study was he Coopex® grains storage powder containing Permethrin as active ingredient at

the concentration of 0.05%$^{w}/_{v}$ and at the rate of 0.5kg per tone. The Coopex® powder was properly mixed with rat pellets made by Pfizer Animals Feeds Plc, Kaduna at the above rate and stored between 7 and 14 weeks.

Animals used for this study was thirty male Albino (Wistar) rats of average weight of 140gm and age of ten weeks and were grouped as A, B, and C of 10 rats each. The animals in groups A were used as the primary control group. The second group (B) and the third group (C) were used as the test groups and as such were fed with the experimental diet after seven weeks of storage while animals in group C were fed with the experimental diet after fourteen weeks of storage. The control group (A) was fed with the normal rat pellets. All the animals were allowed free drinking water *ad libitum*. The feeding period lasted for fourteen weeks. The rats were weighed before the commencement of the feeding experiments and thereafter weighed weekly. The daily food consumption rate was recorded.

At the end of the fourteenth week of feeding, for groups B and C respectively, the animals were sacrificed and a mid-line incision made in the ventral body wall. About 2-3cm lengths of jejunum were exercised and rinsed in normal saline and fixed in 10% neutral formalin. The tissues were processed using the Histokinetic tissue processor. The sections were embedded in paraffin, sectioned and stained using Haematoxylin and Eosin (H and E) method and Periodic Acid Schiff (PAS) method according to Drury and Wallington, (1973).

Morphometric Analysis

Twenty villi from the jejunum of each of the 3 groups A, B and C animals were seected at random, according to the randomised lock design for Morphometry of Mayhew, (1988), and Abbas, et. al., (1989). The length of the villi, the mucosal length and the stromal dimensions were measured using a Leitz Dialux microscope at the magnificant of 10 x 10 and micrometers both stage and eye-piece components according to the specialification of Abbas, et. At., (1989).

Estimates of surface area of the villi over the surface area of the primary mucosa S(V)/S(PM), obtained from each gorup of animals were used to calculate group menas and their coefficient of variation according to Mayhew, (1988), through which the surface amplification due to the villi was determined for each of the groups.

Statistical Analysis

Different parameters from the groups of animals were compared using their mean and standard deviation (SD). The students' t-test was used for testing the level of significance. A p-value less than 0.05 were considered significant. One- way Analysis of Variance (ANOVA) was used for comparing the means while the Multiple Range Test was used to find the Least Significant Difference (LSD) between the groups.

RESULT

The effect of pesticide residue on the mean change in body weight in the three groups is shown in table 1. The results showed that the mean change in weight of the control group (A) was greater than those of group C which in turn had a mean change in body weight greater than those of group B. the mean change in body weight of 89.67$\pm$ 22.05g; 55.33 $\pm$ 14.03g and 66.64$\pm$ 18.47g for groups A, B and C respectively. The mean change in body weight of the control group was significantly greater than those of the experimental groups B and C ($P<0.05$) while the mean change in body weight of the two tests groups were also statistically different ($p<0.05$). At the beginning of the experiment, the body weights of the rats in the three groups were not significantly different from each other.

The mean weights of food consumed per group are shown in Table 2. The result showed that the rats consumed their food at the rate of 141.46 $\pm$ 8.56g; 97.28 $\pm$ 9.8g and 111.64 $\pm$ 13.36g for groups A, B and C respectively. The rate of food consumption in the three groups were significantly different ($p<0.05$).

The surface amplification factors due to jejunal villi estimated by transverse sectioning approach for the three groups are shown in Table 3. The result showed that the surface amplification factors are 6.66 + 0.57; 3.82 $\pm$ 0.37 and 4.53 $\pm$ 0.55 for groups A, B and C respectively. This means that the jejunal villi amplify the mucosal surface area 6 to 7 times; 3 to 4 times and 4 to 5 times for groups A, B and C respectively. The amplification factor differs significantly

between the control and the test groups and in-between the test groups ($p<0.05$).

The result on the villous height, crypt depth and stromal dimensions are also shown in Table 3. The villous length are 498.75 ± 29.51um; 408.25 ± 26.72um and 149.0 ± 21.25um for groups A, B and C respectively. The stromal cross-section for groups A, B and C are 82.75 ± 22.2um; 102.5 ± 31.05um and 106.75 + 32.42um respectively. The length of the villi differs significantly between the three groups ($p<0.05$). The difference in the crypt depth was significant between groups A and B; and groups B and C ($P<0.05$), while the difference between groups A and C was not significant. The difference in the stromal cross-section of the three groups was not significant ($p<0.05$).

DISCUSSION

The results showed that the control group have greater change in body weight that the test groups. This may be, due to their ability to selectively absorb more protein than the test groups (Timbrell, 1982; Scales, 1993). The test groups have reduced body growth, which may be a direct result of non-palatability of the food due to the formation of flavour reversion compounds by the pesticide residues (Kwanashie, 1990). These pesticide residues could have caused decreased rate of food consumption in these animals thus resulting in decreased weight by the test animals when compared with the control group.

The reduced quantity of food consumed and body growth could also be attributed to the inability of the small intestine of the test animals for effective nutrient absorption. This may be caused by the oxidative toxicity of the mucosa of the small intestine bearing the villi, which form the main organ responsible for the absorption of nutrients (Guyton & Hill, 1996; Pumford and Halmes, 1997). The higher body weight of the control group showed that their nutrient absorption are effective while those of the test groups were defective which may be due to the presence of pesticide residues in the test diet of which the group B animals were affected more than the group C animals. This may be interpreted due to the longer period of storage in group C diet while group B had a shorter period of storage before administering the feed to the animals. The result showed that the damage may have been inflicted upon the intestine morphology of the test animals with those of group B being more pronounced due to the short period of storage (Aminu, 1998).

The reduced quantity of food consumed and reduced gain in body weight of test animals also may attributed to the reduction in surface amplications of the intestinal musoca due to the reduction in the length of the villi. The study showed decreased length of the villi in the two test groups, which may result in decreased rate of absorption because of reduction in surface area of the small intestine. This may be as a result of the pesticide residues in the feed of which the level of toxicity differs due to the difference in the storage time which have been shown residual toxicity to pesticides (Scales, 1993; Seth, 1996). The result of this study agrees with Mayhew (1988) who showed that the villi are relatively fixed in number during life but the amplification factor changes during experimental fasting and diabetes.

CONCLUSION

It has been demonstrated that pesticide residues are present in Nigeria foods and drinks. (Kwanashie, 1990). It has been shown that these residues are toxic to tissues of the body but the level of toxicity varies with the level of exposure to the pesticide residues present in the foodstuffs (Seth, 1996; Brimblecombe & Dayan, 1993). The result of this study showed that damage could have been inflicted on the intestinal morphology of the test animals and as such resulted in the morphometric changes in the intestinal mucosa, villi and stroma. This may be due to the toxic products in pesticide residues, which may predispose to reduction in the rates of absorption and growth of the test animals.

These pesticide residues are present in our foodstuffs and in the environment due to the possibilities of non-uniformity of mixing, unwitting repetition of treatment and overdosing by unsupervised, ignorant and illiterate peasant framers (Kwanashie, 1990; Seth, 1996) and when treated foodstuffs are brought to the market prior to the time allowed for the residual effects to reach tolerable level.

Table 1: Mean change in body weight per rat during the feeding period (gm).

Rat No.	Gp A	Gp B	Gp C
1	64.3	60.5	54.2
2	146.3	56.8	53.5
3	87.6	60.7	88.4
4	96.6	77.5	58.6
5	86.7	71.8	89.8
6	79.6	48.8	70.2
7	93.8	58.9	79.8
8	78.9	48.5	32.9
9	75.5	37.2	56.7
10	87.2	32.6	82.3
Total	**896.7**	**553.3**	**666.4**
Mean	89.67	55.33	66.64
S.D	±22.05	± 14.03	± 18.47
Range	(64-147)	(32-78)	(3-90)

Table 2: The mean rate of food consumption (gm) during the feeding period in rats.

Weeks	Gp A	Gp B	Gp C
1	120.7	86.2	97.6
2	141.5	98.4	95.7
3	150.6	89.5	142.2
4	152.7	89.7	99.8
5	149.8	92.6	121.6
6	135.7	91.6	98.5
7	138.5	88.8	110.2
8	132.6	90.3	109.1
9	142.8	107.1	120.7
10	141.9	99.9	98.2
11	139.6	95.6	108.2
12	151.7	101.6	122.8
13	138.8	120.5	119.9
14	143.6	110.2	118.5
Total	1980.50	1361.90	1563.0
Mean	141.46	97.28	111.64
S.D	±8.56	± 9.80	± 13.36
Range	20-153)	(86-121)	(96-142)

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Table3: Small Intestinal Morphometry (Mean, S.D and Range), following the consumption of the control and experimental diets in rats.

Variable	Group A	Group B	Group C
Total Villous Length (um)	9975	8165	8865
Mean	498.75	408.25	443.25
S.D	±29.51	± 26.72	±50.58
Range	(460-560)	(360-460)	(380-510)
Total Crupt Length (um)	3060	2801	2980
Mean	153.0	140.05	149.0
S.D	±22.91	±18.38	±21.25
Range	(125-195)	(120-180)	(120-180)
Total Mucosal Height (um)	651.75	548.30	592.25
Total Stromal Dimension (um)	1655	2045	2135
Mean	82.75	102.25	106.75
S.D	±22.21	±31.05	±32.42
Range	(35-120)	(80 –200)	(35-160)
Total Amplification Factor	133.28	76.44	90.51
Mean	6.66	3.82	4.53
S.D	±0.57	±0.37	±0.55
Range	(5-7)	(3-4)	(3-5)