Original Research Article

Assessment of the Histopathological Effects of Potash on the Hearts of Adult Wistar Rats

ABSTRACT

The histopathological effect of potash was carried out on the hearts of adult wistar rats. As a result of the toxicity cases that had been observed in potash intake. Seventy-five (75) rats were used for the study. The substance administration was given daily for 21 days (3 weeks) and the weights of both the test animal and control was monitored before and after administration of potash. After the administration, the rats were put under light chloroform and the hearts harvested for histological processing. The study revealed that continuous consumption of potash resulted to some varying degrees of distortion and disruption of the cytostructure of the heart, as compared to the control group which show normal cellular and continuous consumption of potash on the heart with that of group D and E appearing more remarkable. Considering the observations in the results of this experiment, it is obvious that in every concentration of the administered potash caused considerable histological abnormalities and cytostructural distortion of the heart, which may be linked to the cytotoxic effects of potash (kanwa) on the heart tissues. The injurious effect of potash due to its continuous use in various homes can be avoided by discouraging excessive use as its accumulation could cause serious damage to body organs.

Keywords: Histopathological, Potash, Wistar, Rats, Heart

1. INTRODUCTION

Potash is the common name for various mined and manufactured salts that contain potassium in water-soluble form. The name derives from "pot ash", which refers to plant ashes soaked in water in a pot, the primary means of manufacturing the product before the industrial era [1]. Today, potash is produced worldwide at amounts exceeding 30 million tonnes per year, mostly for use in fertilizers [1]. Potash has several uses; it is used in cooking as a food tenderizer especially in pulse [2], to curdle milk, in the tanning industries and in the preparation and enhancement of flavor of local beverages and snuffs. However, it has been reported from recent scientific report that there is an increase use of geological mineral substances in human and animal food. And the renewed interest by Nigerian government in solid mineral exploration may possibly explain the reason for the use of naturally occurring inorganic salts for diverse purposes [2]. Potash (especially potassium carbonate) has been used from the dawn of history in bleaching textiles, making glass and from about AD 500, in making soap. Potash was principally obtained by leaching the ashes of land and sea plants. One of the world's largest deposits, 140 to 150 million tons, is located in the Tigray's Dallol area [3]. Potash was one of the most important industrial chemicals in Canada. It was refined from the ashes of broadleaved trees and produced primarily in the forested areas of Europe, Russia, and North America. It is also for an improvement "in the making Pot ash and Pearl ash". Pearl ash was a purer quality made by the ignition of cream of tartar [4].

It is used extensively in ethno-veterinary practices for the treatment of skin diseases and digestive problems. It also serves as a salt lick and mineral supplement in ruminants [5] and used in decoctions for the treatment of reproductive ailments such as retained planta and difficulty in urination. Its medicinal potency has been documented for ailments such as stomach ache, constipation and toothache [5]. The historical use of natron as an aid in childbirth has been well documented. In the kanuri tribe of Borno, it is used to increase uterine contractility and motility for safer childbirth [1]. In the Hausa-Fulani tribe of northern Nigeria, it is used in postnatal care of the puerperium as nursing mothers consume large quantities of potash (about 40g equivalent to 450M of Na) daily in a pap of guinea corn as part of the forty-day postpartum practice in the belief that it increases the quantity and quality of breast milk. Consequently, this has been implicated in the incidence of pre-partum cardiac failure among nursing mothers in this region.

Traditionally, it is used to treat various ailment related to endocrine and reproductive systems such as painful uterus, inducing uterine contraction or abortions, management of retained placenta and postpartum bleeding, infections, infertility, coli pains and treatment of irregular and painful menstruation. One of its folkloric claims is its use as an abortifacient which has not been substantiated scientifically [1]. Potash has also been claimed to be useful in curing cough and to provide relief from toothache, stomach pains, and constipation. Although, some studies have investigated the biochemical and physiological effect of potash but there is still paucity of data concerning it dietary effects in humans [6] [7]. The heart is a hollow muscular organ that pumps blood throughout the blood vessels to various parts of the body by repeated, rhythmic contractions. It is found in all animals with a circulatory system, which includes the vertebrates. The vertebrate heart is principally composed of cardiac muscle and connective tissue. Cardiac muscle is an involuntary striated muscle tissue specific to the heart and is responsible for the heart's ability to pump blood [8].

Various studies have been carried on the effect of potash on various organs of the body. Report has shown that potash present many biochemical changes in the body ranging from mild to severe damage in various organs of the body [1]. Also, nitrate poisoning caused by potassium nitrate (Potash) affect several biochemical parameters and organs such as liver, kidney, heart etc. [6] [7] [9] [10] [11]. Considering the role of the heart as the pumping organ of the body [8]; the heart might be at risk since all ingested substances must be circulated in the body via the blood. Similarly, cases of death have been attributed to the ingestion of toxic substances [12]. Nitrate poisoning of which can occur virtually in all animals has also been linked to potash consumption [13]. Also several acute and chronic health hazards have also been reported to be caused by potash when it is in the nitrate form. Therefore, the need to study the histopathological effect of potash on the heart of adult wistar rats (the animal model).

It is of a known fact that the consumption of potash is quit high and also limited literature exists as regards the effect of potash on various body organs. The increased use of potash has been reported to affect the heart, liver and kidney [6] [7] [9] [10]. Therefore, this study will provide the explorative knowledge on the histopathological effect of potash on the heart of adult wistar rats.

2. MATERIAL AND METHODS

In this study, a total of seventy-five (75) adult Albino Wistar rats of comparable sizes were used for this study. They were divided into six groups with ten (10) rats in group A, B, C, D and E and LD_{50} with 25 rats. Group A served as the control and the rats were given distilled water. Groups B, C, D and E rats were given 0.2g/ml, 0.4g/ml, 0.6g/ml and 0.8g/ml of Potash respectively based on the toxicity levels recorded by previous studies with the last group (F) been the LD_{50} group. The LD_{50} was determined to ascertain the lethal dose of the potash in the study.

The animals were fed with standard rodent food and water *ad libitum* under strict hygienic conditions. They were divided into six (6) groups. LD_{50} and other five groups with 10 rats each and allowed to acclimatize for 7days at room temperature ($25\pm{}^{0}C$), relative humidity (45 to 55%) and 12hours dark/light cycle. The animals were weighed on the first day of the acclimatization period and fed with feed and water given as desired. They were housed in well ventilated labeled wooden cages at the site of the experiment. The cages were designed to secure the animals properly especially from wild animals/insects and cleaned daily.

The substance administration was given daily for 21 days (3 weeks) to enable us have a lengthy time for confirmed observations and the weights of both the test animal and control was monitored before and after administration of potash. After the administration, the rats were put under light chloroform (36mg/kg) and the hearts harvested for histological processing.

Seventy-five (75) adult wistar rats were obtained.

Considerable quantities of Potash were purchased from commercial Market.

2.1 Substance Preparation

The Potash purchased was carefully poured on a clean dry plastic container. From this container it was measured using Electric Balance and packaged in small plastic envelopes and then stored pending usage. The substance preparation process was performed with maximum care in order to avoid any form of contamination.

2.2 Substance Administration

The rats were weighed before the administration of Potash and before they were sacrificed. The administration of Potash was performed by given orally as follows:

- **Group A** (Control) received 150g of normal feed (growers' mash) and distilled water daily for 21days with no administration of Potash.
- **Test group B** received 0.2g/ml of Potash plus 150g of feed daily and water was given ad libitum for 21 days.
- **Test group C** received 0.4g/ml of Potash plus 150g of feed daily and water was given ad libitum for 21 days.
- **Test group D** received 0.6g/ml of potash plus 150g of feed daily and water was given ad libitum for 21 days.
- **Test group E** received 0.8g/ml of potash plus 150g of feed daily and water was given ad libitum for 21 days.

Hence, For the purpose of this study, graded doses of potash were mixed with 1ml of distilled water and administering it orally for 21 days after two weeks of acclimatization.

2.3 Sample Collection and Analysis

The weight of the experimental animals was measured before and after acclimatization and similar weight measurements were done at the end of the administration and the average weight recorded accordingly. The heart of each rat were obtained at the end of the administration (3 weeks) under light chloroform anesthesia and fixed in 10% formalin for histological processing.

The sections were examined under a light microscope and photomicrographs of each group were taken. The photomicrographs are then used to interpret the results of all the groups.

2.4 Data Analysis

The obtained data were then subjected to statistical analysis using SPSS (version 17). The test groups' values were compared with the values of the control group using ANOVA at 95% level of confidence.

3. RESULTS

3.1 Results on Weight

Table 1 presents the body weight changes of rats fed with potash at various intervals. The results showed that the weight of the control increased during the period of the study. However, the weight of the rats fed with potash decreased significantly in all the groups. The mean and standard deviation value of body weight of rats in all groups before and after the period of potash administration was found to be;

Table 1: Body Weight Changes Of Rats Administered With Potash At Various Intervals

Weight (g)	Control (n=10)	B (0.2g/ml) (n=10)	C (0.4g/ml) (n=10)	D (0.6g/ml) (n=10)	E (0.8g/ml) (n=10)	F	*P*
WBPA	200±12.50	200±9.50	225±36.25	201±25.50	248±32.45	4.396	0.016 (S)
WAPA	228±11.33	199±7.95	223±24.12	200±24.00	251±27.32	0.779	0.519 (S)

Key:
WBPA: Weight Before Potash Administration,
WAPA: Weight After Potash Administration
n: Number of sample; *P* – Value (p<0.05): Significant; S: Significant

3.2 Results on Observation

Table 2 presents the behavioural observation of the wistar rats in the study. In the feeding, the control rats fed well and normal but the test rats had loss of appetite. The control rats passed out normal stool while the test rats passed out greyish dark colour faeces. The test rats were losing hairs as the study progresses while the control groups were normal. The test rats become inactive as the study progress while the control groups were active.

Table 2: Showing Observations during the Study

Observations	Control	Group B (65mg/ml)	Group C (130mg/ml)	Group D (162mg/ml)	Group E (194mg/ml)
Feeding	Normal	Loss of appetite	Loss of appetite	Loss of appetite	Loss of appetite
Stool passage	Normal	Greyish dark colour	Greyish dark colour	Greyish dark colour	Greyish dark colour
Skin	Normal	Loosing hair	Loosing hair	Loosing hair	Loosing hair
Activity	Very Active	Inactive	Inactive	Inactive	Inactive

3.3 Results on Histological Observations

There were histological changes in the test tissue sections (B, C, D and E) and the changes observed were dosage dependent. Prominent histo-pathological features include the presence of myocardial fibres with characteristic pale staining region in group B and C (figures 2 and 3), myocardial fibers with characteristic pale staining region and some pyknotic nuclei in group D (figure 4) and myocardial fibers with mild vacuolations and myocardial fibres with scanty nuclei population in group E (figure 5). The observed changes are represented in figures 2 – 5.

Table 3: Histological Observations of the effect of Potash on the Heart of Wistar Rats

Histological effect	GROUP A CONTROL					GROUP B (0.2g)					GROUP C (0.4g)					GROUP D (0.6g)					GROUP E (0.8g)				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Normal histology	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Myocardial fibres with characteristic pale staining region.	-	-	-	-	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Myocardial fibres with characteristic pale staining regions.	-	-	-	-	-	-	-	-	-	-	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Myocardial fibres with characteristic pale staining region and some pyknotic nuclei	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Myocardial fibres with mild vacuolations and myocardial fibres with scanty nuclei population.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++++	++++	++++	++++	++++

KEY:
- = Negative; ++ = Mild.
+++ = Moderate; ++++ = Severe

3.4 Micrographs on Histomorphology of the Heart Tissue

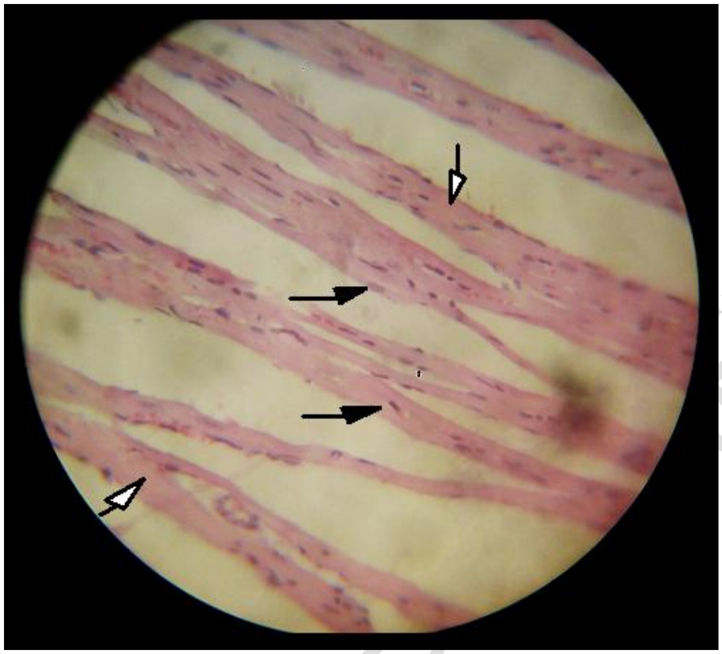

Figure 1: Photomicrograph of group A (Control) Heart tissue section (H&E x400) showing intact heart tissue architecture with distinct myocardial fibres (white arrow) and nuclei (black arrow)

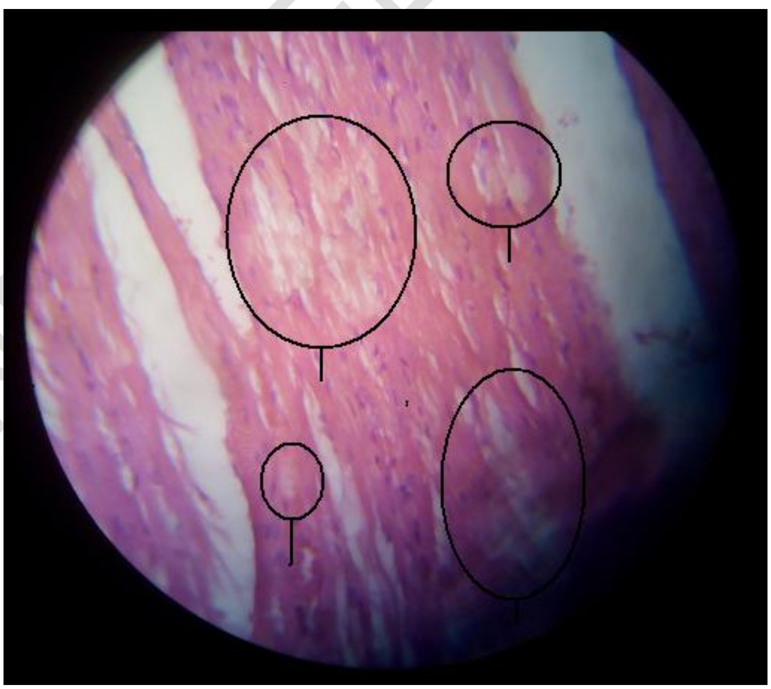

Figure 2: Photomicrograph of group B Heart tissue section (H&E x400) showing myocardial fibres with characteristic pale staining region (encircled)

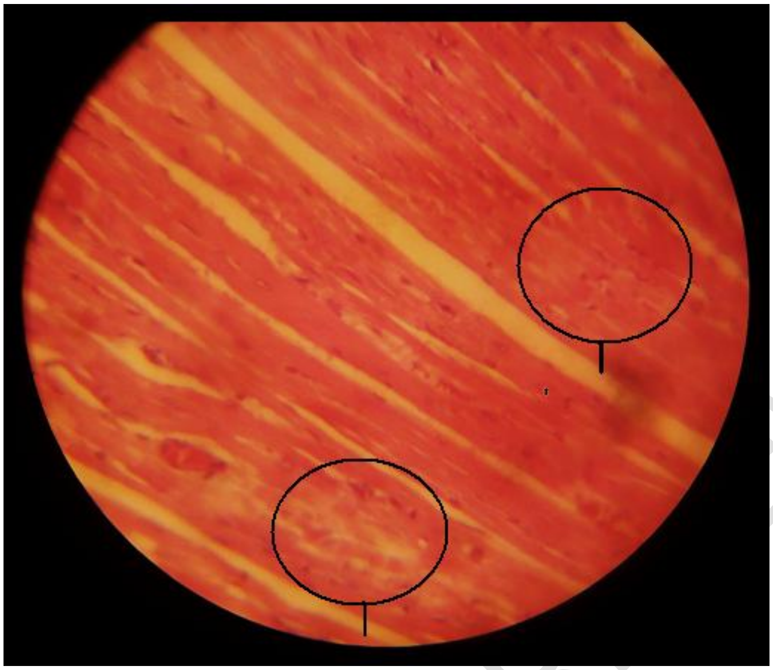

Figure 3: Photomicrograph of group C Heart tissue section (H&E x400) showing also myocardial fibers with characteristic pale staining regions (encircled).

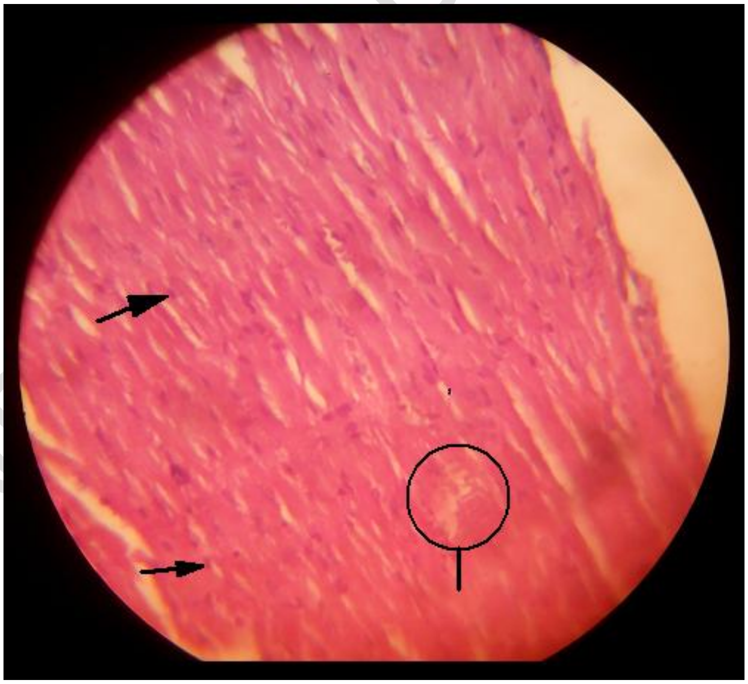

Figure 4: Photomicrograph of group D Heart tissue section (H&E x400) showing myocardial fibers with characteristic pale staining region (encircled) and some pyknotic nuclei (arrows)

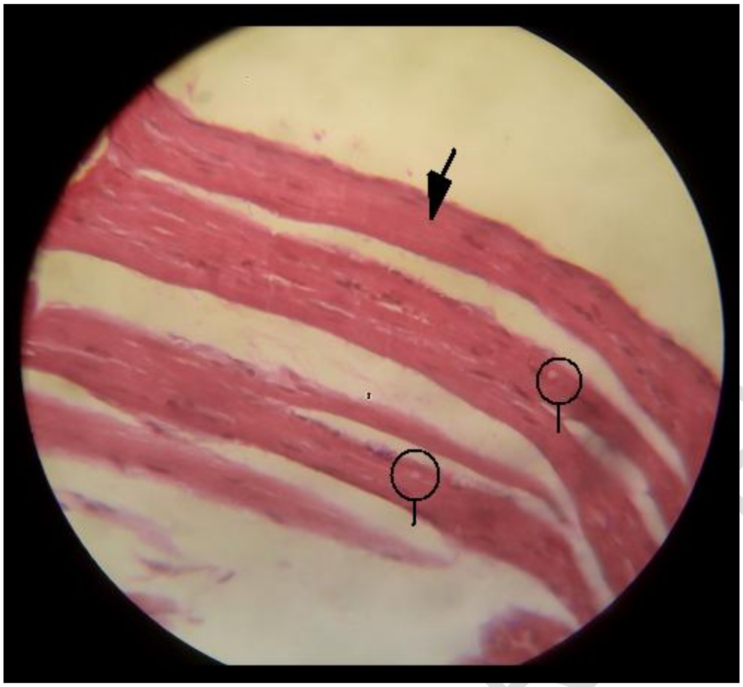

Figure 5: Photomicrograph of group E Heart tissue section (H&E x400) showing myocardial fibers with mild vacuolations (encircled) and myocardial fibres with scanty nuclei population.

4. DISCUSSION

In the feeding, the control rats fed well and normal but the test rats had loss of appetite. The control rats passed out normal stool while the test rats passed out greyish dark colour faeces. The test rats were losing hairs as the study progresses while the control groups were normal. The test rats become inactive as the study progress while the control groups were active.

There were histological changes in the test tissue sections (B, C, D and E) and the changes observed were dosage dependent. Prominent histo-pathological features include the presence of myocardial fibres with characteristic pale staining region in group B and C (figures 2 and 3), myocardial fibers with characteristic pale staining region and some pyknotic nuclei in group D (figure 4) and myocardial fibers with mild vacuolations and myocardial fibres with scanty nuclei population in group E (figure 5).

The study revealed that continuous consumption of potash (kanwa) resulted to some varying degree of distortion and disruption of the cytostructure of the heart, as compared to the control group which show normal cellular and architectural integrity. This finding agree with the reports of Ochei *et al.,* [17], Verma *et al*.,[14] as well as Sreelatha and Padma, [15]. This study does not agree with the report of Bharali *et al.,*[16] whose findings showed that berberine did not prevent histopathological damage and ultrastructure perturbation caused due to isoproterenol-induced myocardial infarction.

The result obtained in this experiment is probably due to the continuous consumption of potash (kanwa) on the heart with that of the group D and E appearing more remarkable. It demonstrates that potash (kanwa) consumption may not be as harmless as generally believed. The structural changes in the heart observed in this experiment could be associated with functional changes that may be detrimental to the health status of the animals. Although the actual mechanism by which potash induced cellular degeneration observed in this experiment is unknown and needs further investigation.

5. CONCLUSION

The aim of using potash on experimental rats was to determine the effects of its repeated use on heart tissues as it is one of the major food supplement in most rural areas in Nigeria and other parts of West African countries. Considering the observations in the results of this experiment, it is obvious that in every concentration of the administered potash caused considerable histological abnormalities and cytostructural distortion of the heart, which may be linked to the cytotoxic effects of potash (kanwa) on the heart tissues. If can also be inferred from the results of this experiment that the distortion of the cytoarchitecture of the heart tissues is associated with functional changes that may have been detrimental to the health status of the animals as a result of potash interference on the heart tissues. However, it has been reported that the extent of severity of tissue damage of a particular compound as toxicant depends on its toxic potentiality on the tissues of organisms. More so, susceptibility to chemical injury varies greatly in the tissues and cells of the same animal. It is sometimes greater in different animal groups. In addition, the location of the major damage may be determined by the mode of action of the chemical. The mode of action of each poison and the pattern of tissue vulnerability has been well defined and the toxic level of each agent at which a fairly standard distinctive pattern of tissue damage has been studied. The result also showed that potash acted as cytotoxic substance to the heart tissues due to its degenerative effects which were seen even at the lowest concentration. The level of potash consumed in various homes however may not be toxic if not taken continuously as indicated, that the effects of potash to the heart tissues are dosage or concentration dependent.

The injurious effect of potash due to its continuous use in various homes can be avoided by discouraging excessive use as its accumulation could cause serious damage to body organs. More research to ascertain the amount that is safe for consumption should be carried out.

ETHICAL APPROVAL

The Ambrose Alli University Ethics Committee gave approval on the use of animal subjects, with the approval number: AAUEC/FBMS/FMLS/DHMA/2018/98/00452.
All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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