Original Research Article

Histopathological Effects of Potash on the Hearts of Adult Wistar Rats

ABSTRACT

The effect of potash on the hearts of adult Wistar rats was studied histopathologically. As a result of the potash poisoning cases that had been documented. A total of 75 rats were used in the experiment. The drug was administered daily for 21 days (3 weeks), and the weights of both the test animal and the control animal were measured before and after the potash administration. The rats were put under mild chloroform after the injection and their hearts were removed for histopathological analysis. In comparison with the control group, that also showed normal cellular and constant consumption of potash on the heart with no distortion or disruption of the cytostructure, the research demonstrates that constant consumption of potash occurred in different degrees of distortion and disruption of the heart's cytostructure, as compared with the control group, that also displayed normal cellular and nonstop consumption of potash on the heart, with group D and E appearing further remarkable. Given the findings of this study, it's clear that every dose of potash provided resulted in significant histological abnormalities and cytostructural deformation of the heart, which could be attributed to the cytotoxic effects of potash (kanwa) on heart tissues. Potash's deleterious effect should be avoided by avoiding excessive use, as its accumulation can cause major damage to body organs.

Keywords: Histopathological, Potash, Wistar, Rats, Heart

1. INTRODUCTION

Potash is the normal name for different mined and made salts that contain potassium in water-dissolvable structure. The name derives from "potash", 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 reports 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 (particularly potassium carbonate) has been utilized from the beginning of history in dying materials, making glass and from about AD 500, in making cleanser. Potash was primarily acquired by draining the cinders of land and ocean 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 main modern synthetic substances in Canada. It was refined from the cinders of broadleaved trees and created fundamentally in the forested areas of Europe, Russia, and North America. It is also for an improvement "in the making Potash and Pearl ash”. Pearl ash was a purer quality made by the ignition of cream of tartar [4].

It is utilized broadly in ethno-veterinary practices for the treatment of skin sicknesses and stomach-related issues. 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 use of natron as a birthing aid has been well recorded throughout history. It is utilised by the kanuri tribe of Borno to improve uterine contractility and motility in order to have a safer childbirth [1]. It is used in postnatal care of the puerperium in the Hausa-Fulani tribe of northern Nigeria, where nursing mothers consume large amounts of potash (about 40g equivalent to 450M Na) daily in a pap of guinea corn as part of the forty-day postpartum practise in the belief that it tends to increase the quantity and quality of breast milk. As a result, the incidence of pre-partum cardiac failure among nursing mothers in this region has been linked to this.

Traditionally, it is used to treat various ailments related to endocrine and reproductive systems such as painful uterus, inducing uterine contraction or abortions, management of retained placenta and postpartum bleeding, contaminations, fruitlessness, colic agonies and, treatment of sporadic and difficult monthly cycle. 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 providing relief from toothache, stomach pains, and constipation. Although some studies have investigated potash's biochemical and physiological effect of potash there is still a paucity of data concerning its dietary effects in humans [6] [7]. The heart is a hollow muscular organ that uses regular, rhythmic contractions to pump blood across the blood arteries to various regions of the body. It can be present in any species that has a circulatory system, including vertebrates. Cardiovascular muscle and connective tissue make up the majority of the vertebrate heart. Cardiac muscle is a striated muscular tissue that is found only in the heart and is important for the heart's capacity to pump blood [8].

Various studies have been carried out 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) affects 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 been reported to be caused by potash when it is in nitrate form. Therefore, the need to study the histopathological effect of potash on the heart of adult Wistar rats (the animal model).

It is a known fact that the consumption of potash is quite 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 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 groups 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 built to keep the animals safe, especially from wild animals and insects, and they were cleaned on a daily basis.

The substance administration was given daily for 21 days (3 weeks) to enable us to have a lengthy time for confirmed observations and the weights of both the test animal and control were 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 the commercial Market.

2.1 Substance Preparation

Potash was purchased and painstakingly put over a perfectly dry plastic holder. It was measured with an Electric Balance and wrapped in little plastic envelopes before being stored pending use. To avoid contamination, the drug production process was carried out with extreme caution.

2.2 Substance Administration

The rats were weighed before and after they were given Potash and slaughtered. 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 this study, graded doses of potash were mixed with 1ml of distilled water and administered 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 was recorded accordingly. The heart of each rat was 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

Following that, the data was subjected to statistical analysis using SPSS (version 17). The test groups' values were compared to the control group's values using ANOVA at a 95% confidence level.

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 samples; *P-Value* ($p<0.05$): Significant; S: Significant

3.2 Results on Observation

Table 2 presents the behavioral observation of the Wistar rats in the study. In the feeding, the control rats fed well and were normal but the test rats had a loss of appetite. The control rats passed out normal stool while the test rats passed out greyish dark color feces. The test rats were losing hair as the study progresses while the control groups were normal. The test rats become inactive as the study progressed 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 color	Greyish dark color	Greyish dark color	Greyish dark color
Skin	Normal	Losing hair	Losing hair	Losing hair	Losing 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 fibers with characteristic pale staining region in groups 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 fibers 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 fibers with characteristic pale staining region.	-	-	-	-	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Myocardial fibers with characteristic pale staining regions.	-	-	-	-	-	-	-	-	-	-	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Myocardial fibers with characteristic pale staining region and some pyknotic nuclei	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Myocardial fibers with mild vacuolations and myocardial fibers with scanty nuclei population.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++++	++++	++++	++++	++++

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

3.4 Micrographs on Histomorphology of the Heart Tissue

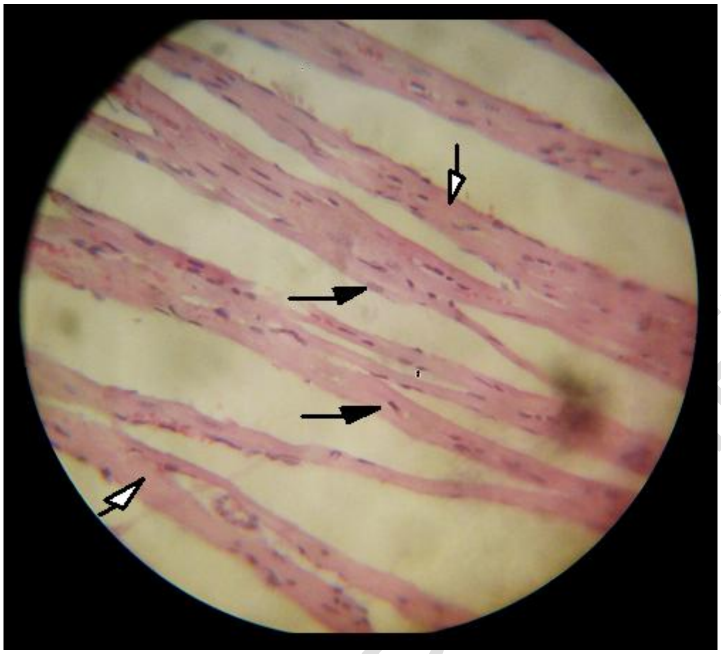

Figure 1: Photomicrograph of group A (Control) Heart tissue section (H&E x400) showing intact heart tissue architecture with distinct myocardial fibers (white arrow) and nuclei (black arrow)

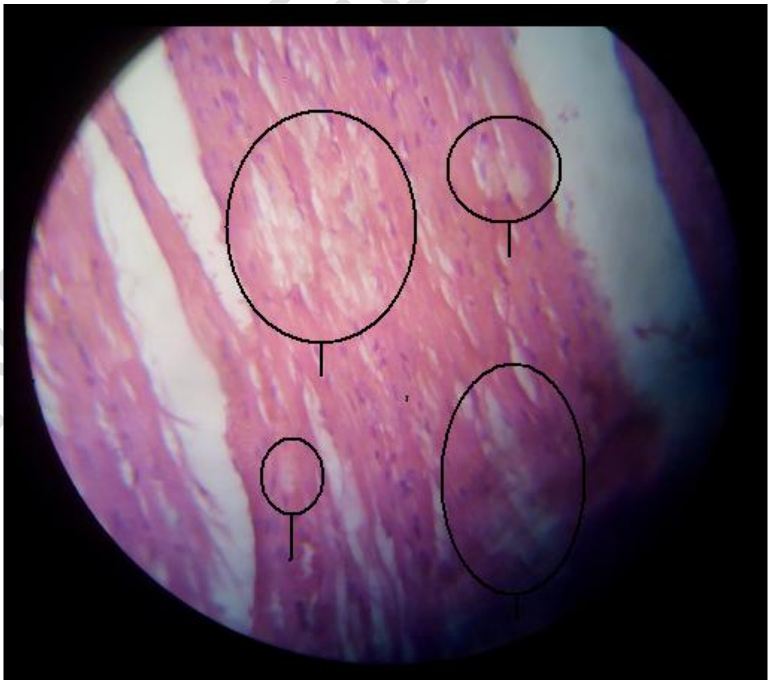

Figure 2: Photomicrograph of group B Heart tissue section (H&E x400) showing myocardial fibres with characteristic pale staining region (encircled)

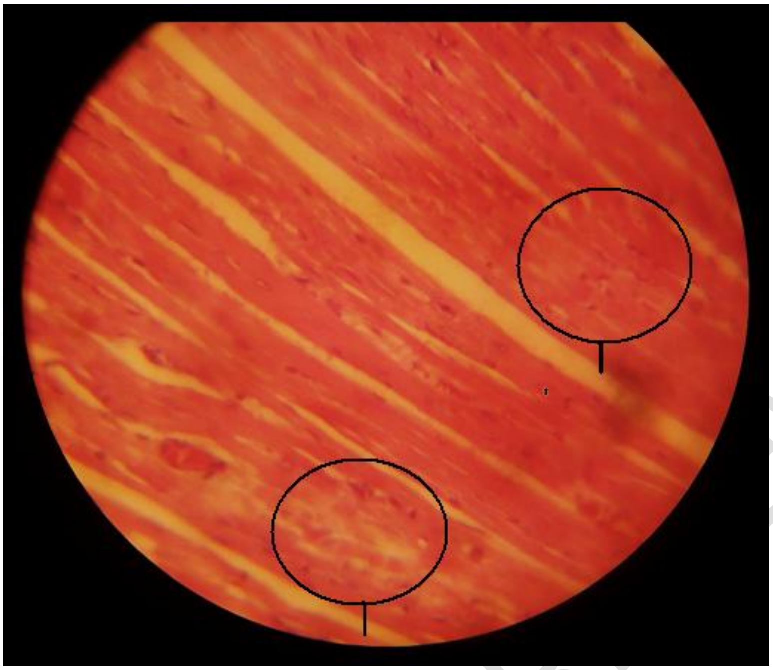

Figure 3: Photomicrograph of group C Heart tissue section (H&E x400) showing also myocardial fibers with characteristic pale staining regions (encircled).

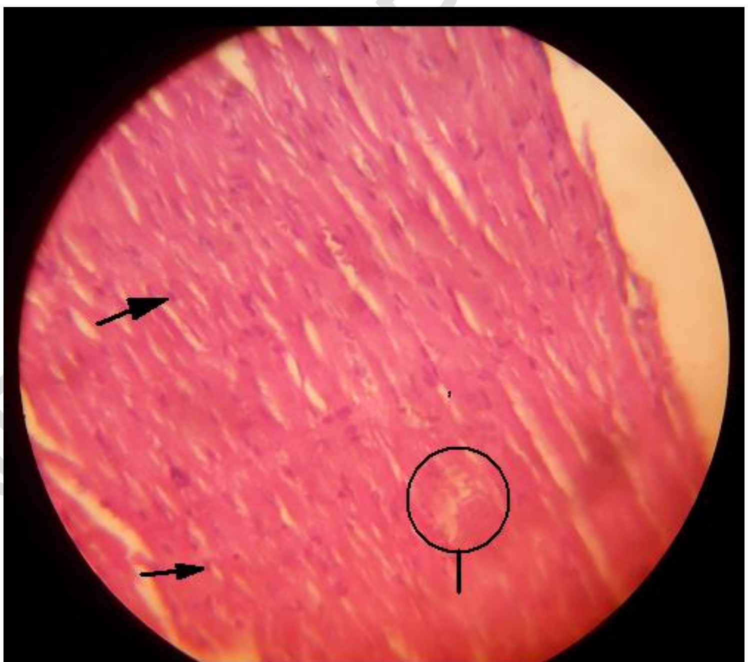

Figure 4: Photomicrograph of group D Heart tissue section (H&E x400) showing myocardial fibers with characteristic pale staining region (encircled) and some pyknotic nuclei (arrows)

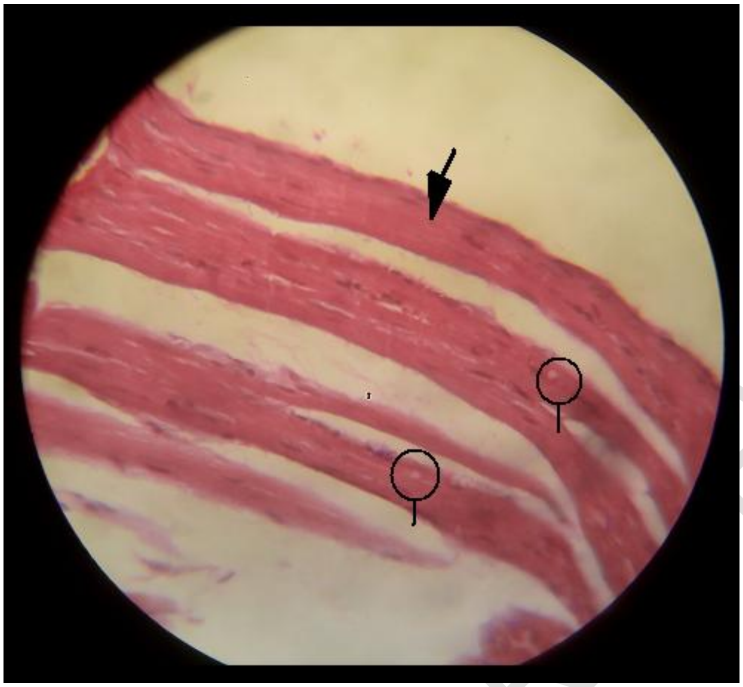

Figure 5: Photomicrograph of group E Heart tissue section (H&E x400) showing myocardial fibers with mild vacuolations (encircled) and myocardial fibers with scanty nuclei population.

4. DISCUSSION

In the feeding, the control rats fed well and were normal but the test rats had loss of appetite. The control rats passed out normal stool while the test rats passed out greyish dark color feces. The test rats were losing hair as the study progresses while the control groups were normal. The test rats become inactive as the study progressed 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 fibers with characteristic pale staining region in groups 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 fibers with scanty nuclei population in group E (figure 5).

In comparison to the control group, which showed normal cellular and architectural integrity, continued use of potash (kanwa) resulted in varied degrees of deformation and disruption of the heart's cytostructure. This finding agrees 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 effect of continuous consumption of potash (kanwa) on the heart is likely to be the cause of the results obtained in this experiment, with the effects of groups D and E being more noticeable. It shows that potash (kanwa) usage may not be as safe as previously thought. The anatomical abnormalities in the heart that were identified in this experiment could be linked to functional changes that could be harmful to the animals' healthAlthough the exact mechanism of potash-induced cellular degeneration revealed in this experiment is unknown, more research is needed.

5. CONCLUSION

Potash was used on experimental rats to investigate the effects of repeated use on cardiac tissues because it is a common meal supplement in the majority of Nigeria's rural areas, as well as those in neighboring West African countries. Considering the observations in the results of this experiment, it is obvious that every concentration of the administered potash caused considerable histological abnormalities and cytostructural distortion of the heart, this could be due to the cytotoxic effects of potash (kanwa) on cardiac tissue. The results of this experiment also suggest that, as a result of potash influence on the heart tissues, the distortion of the cytoarchitecture of the heart tissues is connected with functional abnormalities that may have been deleterious to the animals' health. However, it has been noted that the level of tissue damage caused by a given molecule as a toxicant is determined by its toxic potentiality on organism tissues. Susceptibility to chemical harm varies widely even within the same animal's tissues and cells. Different animal groups have higher levels of it at times. The location of the greatest damage may also be influenced by the chemical's method of action. Each poison's method of action and pattern of tissue vulnerability have been clearly characterized, as has the hazardous dose of each substance at which a reasonably standard test can be performed distinctive pattern of tissue damage has been studied. Each poison's method of action and pattern of tissue vulnerability have been clearly characterized, as has the hazardous dose of each substance at which a reasonably standard test can be performed. 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.

Potash acted as a cytotoxic material to the cardiac tissues, as evidenced by its degenerative effects, which were visible even at low concentrations. More research to ascertain the amount that is safe for consumption should be carried out.

ETHICAL APPROVAL

The Ambrose Alli University Ethics Committee approved 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”

Consent

As per international standards or university standards, patients’ written consent has been collected and preserved by the author(s).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research and country. There is 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 the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

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