Evaluation of the Effect of Some Marketed Herbal Cosmetics in Port Harcourt on Renal Parameters of Rabbits

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Authors' contributions

This work was carried out in collaboration among all authors. Author ESA designed the study, author EON wrote the protocol, author HB wrote the first draft of the manuscript. Authors INT and ESA managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Article Information

Original Research Article	

ABSTRACT

Aim: The aim of this study was to evaluate the effects of three commonly marketed herbal cosmetics in Port Harcourt on the renal parameters in rabbits.
Study Design: This study is an experimental study.
Place and Duration of Study: This study was carried out at Animal House, Applied and Environmental Biology Department, Rivers State University, Port Harcourt, Rivers State, Nigeria, between April 2020 and November 2020.
Methodology: A total of 48 rabbits were used for the study. They were divided into four groups with twelve rabbits in each group. Four rabbits from each group were treated for thirty, sixty and ninety days respectively. All the rabbits were given feed and tap water ad-libitum. Using the Organisation for Economic Co-operation and Development (OECD) guideline for volume selection, 0.5ml/kg of Samples A, B, C and D were each applied to 5cm by 5cm scrapped dermal Forsa of the rabbits in each group every morning for the respective treatment periods stated above. At days

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thirty, sixty and ninety, respectively, four rabbits from each group were sacrificed under chloroform anaesthesia. Blood samples were collected from the rabbits at intervals, 30days, 60days and 90days. The kidneys were harvested at 90 days from the rabbits. The blood was collected to test sodium, potassium, chloride, calcium, urea, creatinine, KIM-1 and kidney for histological analysis. GraphPad Prism v.7.0 was used for statistical analysis and p values less than 0.05 were considered statistically significant.

Results: The results showed that Potassium level was significantly higher *(p<.05)* from day 30 for group A compared to control, group B and group C. This is attributed to group A having a higher level of cadmium and arsenic compared to the other groups. The urea and creatinine result for group A was significantly higher by day 30 *(p<.05)* compared to control with group B and group C. Whereas calcium became significantly lower at day 60. With chloride significantly higher *(p<.05)* at day 60. Early signs of toxicity to the Kidney were identified from the significant effect on the urea, creatinine, KIM-1 and histology results. The continued use of these products contaminated by these heavy metals will release them slowly into the body of recipients and which will invariably damage the kidney.

Conclusion: The continued use of these products contaminated by these heavy metals will release them slowly into the body of recipients and which will invariably damage the kidney. Early signs of toxicity to the Kidney were identified from the significant effect on the urea, creatinine, KIM-1 and histology results.

1. INTRODUCTION

Cosmetics are products used to improve or change the physical looks of the human face and skin by simply applying them on the mentioned areas. Cosmetics are generally a mixture of different chemical or natural compounds, produced from either synthetic or plant sources. Examples of cosmetics include lipsticks, eye shadows, foundation, skin cleansers, lotions, cream, shampoo, conditioner, hair styling products, gels, hair spray, perfume, and cologne [1]. There are two major types of cosmetic products, which are classified based on the source of the raw material used for production. They include synthetic and herbal cosmetics. When chemical compounds are used in the creation of the beauty product, it is known as a synthetic cosmetic. While herbal cosmetics involves the use of parts or whole of plant material in the manufacturing of the beauty product [1]. There is an all-encompassing variety of herbal cosmetics that are manufactured and commonly used for everyday purposes. These herbal cosmetics, such as conditioners, soaps, face wash, shampoo are produced from a plant source. The best thing about these cosmetics is that when it is made from plant sources, it is majorly believed that the original content in the plant does not have any adverse reactions to the human body [2]. Instead, it enhances the body by providing it with beneficial vitamins and minerals contained in the plant. Hence, herbal cosmetics contain lots of vitamins and minerals [2].

The global herbal cosmetics industry is presently estimated to be higher than US$10 billion and growing at an alarming rate of about three to four per cent per annum [3]. There is a high demand for this type of cosmetic in the form of creams, conditioners, lipsticks, oils, shampoos, and gels. The highest need for them is in Europe, followed by Asia. The upsurge in the use of herbal cosmetics around the world is because they are safer for health than the synthetic ones [3-4]. Herbal cosmetics is the new trend in the field of fashion and beauty due to the belief that it is safer than the synthetic ones. Although there are cases of reports involving severe adverse events after using some naturals cosmetic. In a lot of these cases, the toxicity is due to contaminants and pollution. Herbal cosmetics can therefore carry a risk of adverse effects if not adequately tested. Scientific research has also now discovered that not all herbal products are safe, as per general belief, and that some are quite toxic, having several adverse effects. Toxicants in herbal cosmetics include heavy metals and phytochemicals in high amount [5]. Example of heavy metals include Arsenic, Mercury, cadmium, copper, iron, lead, silver, gallium, and indium etc [5]. Testing for toxicity in herbal products, therefore, is of utmost importance in herbal research. As a result, the quality, safety, and efficacy of herbal products has become an essential concern for both consumers and health authorities throughout the world. Therefore, the aim of this study was to evaluate the effect of some marketed herbal

cosmetics in Port Harcourt on renal parameters of Rabbits.

2. MATERIALS AND METHODS

2.1 Procurement of Herbal Cosmetics

Three (3) types of commonly used herbal hair oils were purchased from a Supermarket in Port Harcourt, Rivers state, Nigeria labelled, sample A, B and C, respectively and used for this study. Sample A is Allthingsnatural by Emi herbal oil, sample B Is Kakiva herbal oil and sample C Is Amal botanical herbal oil.

2.2 Experimental Animals

A total of forty-eight (48), Two-month-old, white rabbits (*Oryctolagus cuniculus*) that weighed between 1.2 - 1.5 kg were used for the study. The rabbits were purchased from a breeder in Port Harcourt, Rivers State, Nigeria. The rabbits were weighed and grouped into 4 groups of 12 rabbits each. Group A were treated with Allthingsnatural by Emi herbal oil, group B treated with Kakiva herbal oil, group C with Amal botanical herbal oil and the 4th group treated with deionized water (group D).

2.2.1 Housing and nutrition

The rabbits were kept in a spacious and well-ventilated cage at room temperature, under natural circadian rhythm and allowed to acclimatize for fourteen (14) days. They were housed in standard cages and allowed access to feed (Top Feed Finisher Mash, Sapele, Nigeria) and water *ad libitum* in the animal house. All the animals received humane treatment according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Institute of Health.

2.3 Dosage Calculation of Volume of Oil Used

Using the OECD guideline for volume selection, 0.5ml/kg of the Sample A, B, and C were each applied to 5cm by 5cm scrapped dermal Forsa of the rabbits in each group every morning [6]. Deionized water was applied to the scrapped dermal forsa of the rabbits in the control group. All the rabbits were given feed and tap water ad-libitum.

2.4 Sample Collection and Storage

At days zero, thirty, sixty and ninety, respectively, four rabbits from each group were sacrificed under chloroform anaesthesia. Blood samples were collected from the rabbits at intervals, 30days, 60days and 90days. The kidneys were harvested at 90 days from the rabbits. The blood was collected to test sodium, potassium, chloride, calcium, urea, creatinine, KIM-1 and kidney for histological analysis. All the kidney function parameters were carried out at Nigerian National Petroleum Corporation (NNPC) Clinic, Akpajo, Rivers state, Nigeria while histology was carried out at Rivers State University, Port Harcourt, Rivers State.

2.5 Laboratory Determination of Parameters

2.5.1 Determination of serum urea

To test for urea test tubes were arranged and labelled as Test (T), Standard (S) and Blank (B). In the various tubes $100 \mu l$ of plasma, standard and water was dispensed. 1ml of urease enzymatic reagent was added into the different tubes, mixed gently, and incubated at $37°C$ for 25 minutes. 2.5ml of diluted phenol was added into the different tubes; 2.5ml of hypochlorite was also added to the various tubes. The blue colour formed was read colorimetrically at 520mn.

Calculation: Urea concentration=
$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Standard concentration}$$

2.5.2 Determination of serum creatinine

To test for creatinine test tubes were labelled as T1 (Reagent blank), T2 (Test sample), and S (standard). $180 \mu l$ of working picric reagent and distilled water was dispensed into T1, while $180 \mu l$ of picric reagent and plasma was dispensed into T2, $180 \mu l$ of picric reagent and standard was dispensed into S. The content in each of the test tubes were mixed and incubated at $37°C$ for one minute, followed by the addition of $180 \mu l$ of alkaline reagent into all the tubes. The content of the tubes was mixed thoroughly and incubated at $37 °C$ for 30 seconds. Absorbance (A1) of the first reading was read after 30 seconds while the second reading of the Absorbance (A2) was read after 90 seconds at 520mn against blank.

Calculation: The difference in absorbance reading was calculated with the formula (ΔA= A2 – A1). Then, the ratio of the difference in absorbance for the test sample and the standard multiplied by the concentration of the standard.

2.5.3 Determination of kidney injury molecule

This assay employs the quantitative enzyme immunoassay technique (double-antibody sandwich) to assay the kidney injury molecule.

Principle: The microtiter plate provided has been pre-coated with an antibody. Add standard, sample, and conjugated antibody to wells. After incubation and washing to remove the uncombined enzyme, add chromogen solution and B. The colour of the liquid will change into blue. At the effect of acid, the colour finally becomes yellow. The colour change is measured spectrophotometrically at a wavelength of 450nm. The concentration of kidney injury molecule in the samples is then determined by comparing the optical density (O.D.) of the samples to the standard curve.

2.5.4 Histological analysis

The kidneys were harvested for histological analysis and were fixed in 10% formal saline solution. The organs were dissected, and representative blocks were taken for histological processing each with identifying label in a tissue cassette. The fixed tissue blocks were dehydrated through ascending grades of alcohol, de-alcoholized in xylene, infiltrated and embedded in molten paraffin wax. Sections were cut at 3μm on a rotary microtome. Deparaffinized sections were then stained with the standard haematoxylin and eosin staining technique and the slides mounted in DPX. Sections on the slide were examined and photomicrographs captured with X400 objective lens using the ScopeTek™ device and software v1.3.

2.5.5 Quality assurance

Standard operating procedures (SOPs) were strictly adhered to. Quality control samples were included in the run of the samples. Equipment were properly calibrated. Good laboratory practise was ensured in all processes.

2.6 Statistical Analysis

The experimental data for various renal parameters obtained were analyzed using GraphPad Prism version 7.0 developed by GraphPad Software San Diego, California, USA.

Data are presented as Mean± SD, ANOVA and Tukey's multiple comparison test were used for comparison of mean values of groups that are more than two. Variation in means of parameters was considered statistically significant at *p<.05*.

3. RESULTS AND DISCUSSION

The effect of the herbal products on the renal parameters were assessed by determining the Sodium, potassium, chloride, urea, creatinine, kidney injury molecule-1 and calcium level in the rabbits after thirty, sixty and ninety days of exposure to the different herbal oils. The Potassium level was significantly higher (*p<.05*) from day 30 for group A compared to control, group A and group B (Table 1).

This is attributed to group A having a higher level of cadmium and arsenic compared to the other groups. The urea and creatinine result for group A was significantly higher by day 30 (p<0.05) compared to control with group B and group C. Whereas, calcium became significantly lower at day 60 (Table 2). With chloride significantly higher (p<0.05) at day 60. Similar results of changes in the levels of calcium and creatinine were observed in other studies [7-10].

In these studies, there was a decrease in serum levels of Ca and an increase in creatinine level in comparison to the control group. Although, it was noticed that the serum urea concentration decreased. Group B had the least level of significant amount of change in its renal parameter followed by group C, then group A. The kidney is a major organ affected by cadmium exposure and chronic exposure is mostly by ingestion and dermal route of exposure such as in this study [11]. Frequent exposure to Cadmium contributes to its excess accumulation in kidney, which results in renal damage and nephropathy.

Furthermore, Cd high level reduces calcium absorption, which becomes an impending cause of bone and kidney losses, called Itai-Itai disease. Hence the observable difference in the renal parameters can be attributed to the level of cadmium present in the cosmetics since the cosmetic with higher cadmium content had the greatest significant increase *(p<.05)* as compared to control within the groups. The KIM-1 molecule of all groups showed a significant increase *(p<.05)* from day 30, even when urea and creatinine level in group B and C had yet to show any significant kidney effect as compared to control.

Table 1. Evaluation of blood Na$^+$, K$^+$, CL$^-$, Ca^{2+}, KIM-1, urea and creatinine levels of rabbits exposed dermally to various herbal cosmetics after thirty days

S/N	Samples	Na$^+$ (mmol/L)	K$^+$ (mmol/L)	Cl$^-$ (mmol/L)	Ca^{2+} (mmol/L)	KIM-1 (pg/mL)	Urea (mmol/L)	Creatinine (µmol/L)
1	Control	136.50±0.41^a	4.75 ±0.13^a	109.40±0.91	1.52±0.05	7.25±0.26^a	9.50 ±0.30^a	71.75±18.28^a
2	A	144.10±2.62^c	5.78 ±0.28^b	109.50±1.46	1.49±0.05	15.05±0.17^d	13.23 ±0.75^{bc}	108.30±9.95^{ce}
3	B	140.10±0.87^{bd}	5.32 ±0.36^{ac}	110.50±0.22	1.50±0.01	8.20±0.18^c	9.75 ±0.51^d	95.50±11.03^{ade}
4	C	139.00±1.19^{ad}	5.41 ±0.35^{bc}	109.60±1.21	1.52±0.01	13.23±0.17^b	11.18 ±0.25^c	97.00±0.82^{bcd}
5	F_value	17.06	8.391	1.041	0.8224	1419	45.45	6.781
6	P value	0.0001	0.0028	0.4098	0.5063	<0.0001	<0.0001	0.0063

Values are expressed as mean ± SD. n =4, Mean ± SD of experimental groups with different superscripts are significantly different from each other at p<0.05.

Table 2. Evaluation of blood Na$^+$, K$^+$, CL$^-$, Ca^{2+}, KIM-1, urea and creatinine levels of rabbits exposed dermally to various herbal cosmetics after sixty days

S/n	Sample	Na$^+$ (mmol/L)	K$^+$ (mmol/L)	Cl$^-$ (mmol/L)	Ca^{2+} (mmol/)	KIM-1 (pg/mL)	Urea (mmol/L)	Creatinine (µmol/L)
1	Control	137.60 ±1.06^a	4.59 ±0.22^a	109.00 ±1.33^a	1.53 ±0.03^a	7.03 ±0.75^a	9.40 ±0.29^a	68.25 ±12.50
2	A	145.90 ±5.19^b	11.38 ±0.24^b	120.10 ±3.60^{cd}	1.43 ±0.04^{bc}	19.55 ±1.04^b	19.18 ±0.77^b	119.50 ±9.33
3	B	138.80 ±3.46^a	10.90 ±0.55^b	117.00 ±0.83^{bdf}	1.41 ±0.04^b	10.20 ±1.18^c	11.65 ±3.20^{ac}	101.80 ±11.18
4	C	139.50 ±1.13^a	10.62 ±0.57^b	113.90 ±3.38^{af}	1.46 ±0.01^{bc}	17.90 ±0.64^b	12.33 ±1.27^c	102.50 ±8.10
5	F-value	5.345	221.4	13.26	10.98	17.06	8.391	1.041
6	P value	0.0143	<0.0001	0.0004	0.0009	0.0001	0.002	0.4098

Values are expressed as mean ± SD. n =4, Mean ± SD of experimental groups with different superscripts are significantly different from each other at p<0.05

Table 3. Evaluation of blood Na$^+$, K$^+$, CL$^-$, Ca^{2+}, KIM-1, urea and creatinine levels of rabbits exposed dermally to various herbal cosmetics after ninety days

S/N	Samples	Na$^+$ (mmol/L)	K$^+$ (mmol/L)	Cl$^-$ (mmol/L)	Ca^{2+} (mmol/L)	KIM-1 (pg/mL)	Urea (mmol/L)	Creatininev(µmol/L)
1	Control	140.60 ±3.13	4.77 ±0.35^a	109.70 ±0.76^a	1.53 ±0.04^a	7.53 ±0.70^a	9.25 ±0.61^a	76.50 ±3.42^a
2	A	148.60 ±3.10	14.96 ±1.73^b	124.90 ±3.88^{cb}	1.31 ±0.06^b	27.55 ±0.87^{cd}	22.10 ±1.54^b	130.50 ±3.00^{bc}
3	B	144.90 ±2.49	14.03 ±0.51^b	119.20 ±4.76^b	1.43 ±0.09^a	13.73 ±0.79^{ab}	12.53 ±2.93^{ac}	124.50 ±15.93^b
4	C	145.00 ±4.96	12.25 ±0.95^{bc}	113.90 ±3.63^a	1.42 ±0.03^a	21.03 ±3.06^{bd}	13.55 ±0.87^c	120.50 ±10.21^b
5	F-value	2.989	80.26	13.49	9.31	13.16	39.70	25.73
6	P value	0.0734	<0.0001	0.0004	0.0019	<0.0004	<0.0001	<0.0001

Values are expressed as mean ± SD. n =4, Mean ± SD of experimental groups with different superscripts are significantly different from each other at p<0.05

A, B and C. Histological examination revealed significant changes in all the groups exposed dermally to the extracts after 90 days as compared to the control group normal saline. The Photo micrographic of kidney tissues of all the groups exposed to Sample A, B and C areas showing moderate 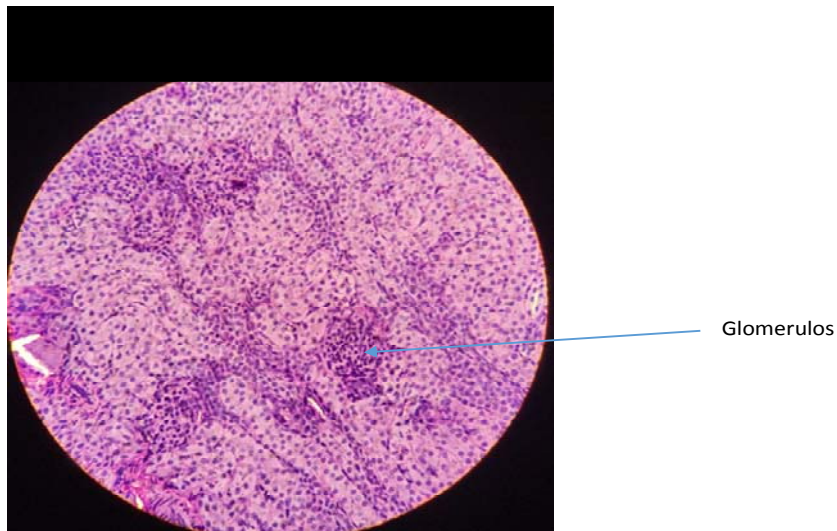Chronic Progressive nephropathy without hyalinization. The different rabbits exhibited features of glomerulosclerosis, thickened basement membrane, renal infarct, tubular basophilia and mononuclear inflammatory infiltrate within the renal parenchyma, while control showing normal morphology.

KIM-1 is a better predictive marker for early kidney damage studies has shown that KIM-1 was shown to increase even in the absence of change in the kidney function parameter. The Photo micrographic of kidney tissues of all the groups exposed to Sample A, B and C showed areas of moderate Chronic Progressive nephropathy without hyalinization (Plates 1-4).

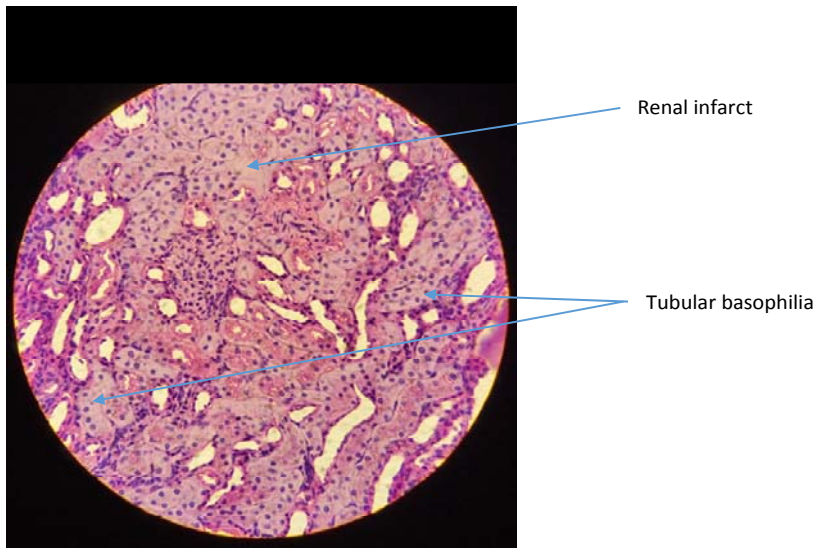**Plate 1. Photo Micrographic Slide of Kidney Organ of Group (Control Saline) H & E X400**

Plate 2. Photo Micrographic Slide of Kidney Organ of Sample A using Allthingsnatural by Emi herbal oil (H & E X400).
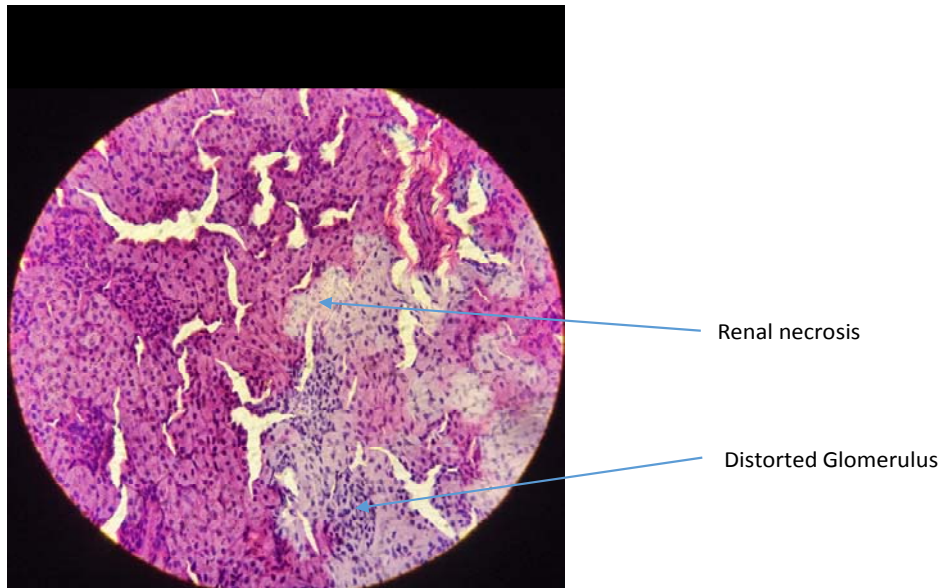
Plate 3. Photo Micrographic Slide of Kidney Organ of Sample B using Amal Botanical herbal oil (H & E X400)
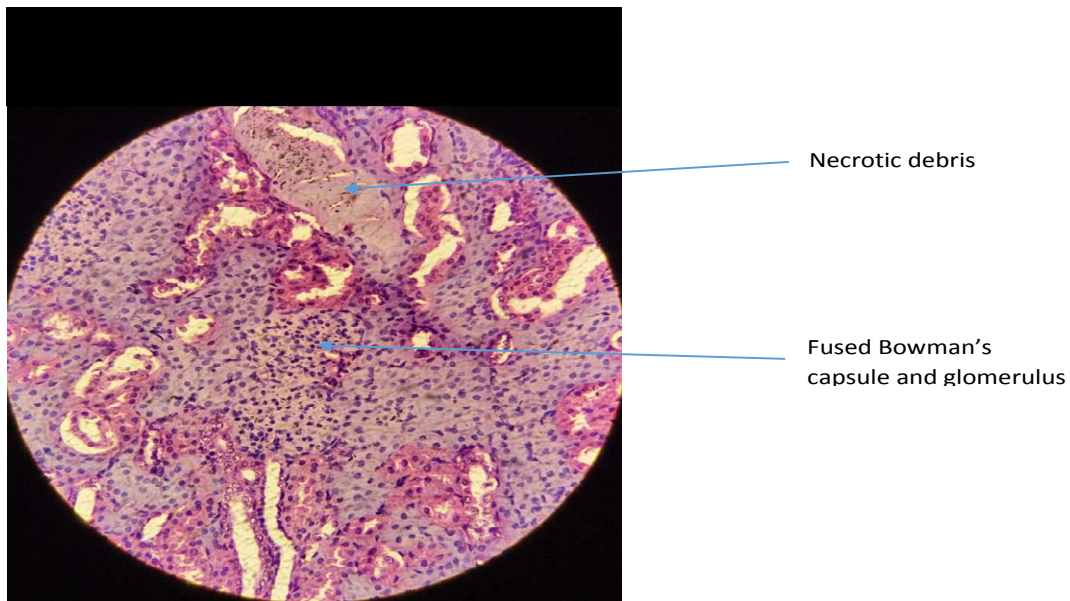

Plate 4. Photo Micrographic Slide of Kidney Organ of Sample B using Kakiva herbal oil (H & E X400).

The different rabbits' tissues exhibited features of glomerulosclerosis, thickened basement membrane, renal infarct, tubular basophilia and mononuclear inflammatory infiltrate within the renal parenchyma. While control showing normal morphology. This is in keeping with the kidney function parameters.

4. CONCLUSION

Early signs of toxicity to the Kidney were identified from the significant effect on the urea, creatinine, KIM-1 and histology results. The continued use of these products contaminated by these heavy metals will release them slowly into

the body of recipients and which will invariably damage the kidney.

DISCLAIMER

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.

CONSENT

It's not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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