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Acute Toxicity Profile Of Topical Administration Of Anti-Arthritic Cream In Wistar Rats

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Abstract
Background: *The use of plant based products as alternative approach to orthodox medicine is on the increase without scientific investigation.*
Aim: *the study investigated acute toxicity studies of topical administration of anti-arthritic cream in Wistar rats.*
Methods*: Eighteen (18) male Wistar rats weighing from 120 g–150 g were selected and used for the study. The rats were divided into three (3) study groups (n=6 rats/group), right hindlimb fur of rats in each group were shaved with a clean razor blade under light anaesthesia for topical administration of anti–arthritic cream for 28 days. The study protocol; Group 1: Control group received 0.2ml saline water Group II: received 1g of the anti–arthritic cream Group III: received 1g of the cream base.and they were fed everyday with rat chow (standard diet), and had access to water* ad libitum. *Acute toxicity was conducted in accordance with the Organization for Economic Cooperation and Development (OECD, 2001) guidelines*
Results ***A****nti-arthritic cream decrease liver weight, lungs, serum alanine amino asphatase (AST), alanine aminotransferase(ALT), increased creatinine, while cream base increased liver weight and lungs, serum AST ALT, creatinine both the anti-arthritic cream and cream base increased the kidney weight, likewise pancreas and heart.weights, low density lipoprotein (LDL) and high density liporotein(LDH). Anti-Arthritic cream and cream base decreased white blood cell(WBC), also anti-arthritic cream decreased red blood cell(RBC), haemoglobin(HGB) and haematocrit (HCT) but cream base increased RBC but decreased HGB and Hct.*
Conclusion: *The study revealed that topical administration of anti-arthritic cream does not cause any deleterious effects on the body vital organs, since there was no tissue necrosis. Also, caution must be undertaken on the long time use of anti-arthritic cream due to it inability to improve red blood cell and white blood cell counts*
Keyword: *Acute toxicity, Topical application, Anti-arthritic cream, Wistar rat*

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I. Introduction

Herbal medicine in developing countries serves as an alternative natural remedy in primary health care system, because it is cheaper, available, and easily accessible in the communities, while in Africa, it was estimated that 70-80% patients are treated by traditional medicine practitioners with the use of traditional remedies [TM][1].In Ethiopia, it has been estimated that 70% of humans and 90% of livestocks depends on TM[2]. Plant based medicine has a socio-enviromental and cultural integration in the countries of Africa. Plant that humans use in Africa can be found in the household or in the geographical areas where there activity occurs daily and they are part of their ethnic or tribal culture[3]. In the world, about about 25% modern drugs developed from the plants[4], plants synthesis varieties of metabolites and some of which may be beneficial or potentially harmful to mankind [5]. It has also been true that pharmaceutical drugs may be therapeutic at one dose and toxic at other doses. In order to ensure safety, there must be a study to show safety profiles of herbs claimed to be beneficial to humans and the animals before deciding to use them[6]. The selective uptake or accumulation of a particular xenobiotic in a specific tissue or cell, the inhibition of the normal export of a potentially toxic metabolite from a cell to the outside, and the activation of cellular receptors could lead to toxicity [7,8]. Acute and sub-acute toxicity tests are routinely performed for the investigation of natural products or drugs. Acute toxicity test is the first step to determine the adverse effects of substances within 14 days of administration of the dose[9,10].

Liver and kidneys are the primary organs affected by metabolic reactions of toxicants and are useful in predicting toxicity effects of phytotherapeutic products or drugs [3]The liver is the main target for toxic

compounds because of its exposure to foreign substances absorbed in the intestine before reaching into the blood circulation [11, 12] Although toxins may harm the liver, it also detoxifies toxins[13]. Thus, in experimental animals, liver function test would allow to understand the toxic effects, which can be extrapolated for safety if used in humans. Blood parameters are relevant indicators for potential health hazards and have a higher predictive value for toxicity [14, 15]. The calculated red blood cell indices, that is, the mean corpuscular volume (MCV) and the mean corpuscular haemoglobin concentration (MCHC) are used for diagnosis of anemia[16]. Heamatological indices such as packed cell volume (PCV) and hemoglobin (HB) are associated with the total population of red blood cells (RBCs). MCV reflects the size of red blood cells; whereas the mean corpuscular heamoglobin (MCH) and MCHC are used mathematically to define the concentration of heamoglobin [17]. The packed cell volume (PCV) or heamatocrit represents the percentage of RBCs of whole blood volume, which is clinically used to determine anemia[17].These blood biomarkers help to determine toxicity in relation to dose and time-response [18]. Furthermore, the considerations of platelet indices such as mean platelet volume (MPV), platelet distribution width (PDW) and a platelet large cell ratio (P-LCR) are also used to screen toxicity [19,20,21]. *In vitro* platelet toxicity assays can be used to evaluate the predictive values of hypersensitivity reactions against drugs [22]. Increasing or decreasing in platelet counts result in clotting or bleeding abnormalities, respectively [23]. The study therefore evaluated acute toxicity profile of topical administration of anti- arthritic cream in Wistar rats.

II. Materials And Methods

Plant materials

Oils of *Mentha pipera, Zigiber officinale*, *Capsicum frustescen* and *Glycyrrhiza glabra plants* were purchsed from the organic shop at Ojota in Lagos State Nigeria and used for the formulation of anti-arthritic cream

Ethical Approval

Ethical approval was obtained from College of Medicine Health Research Ethics Committee with approve Number CMUL/HREC/10/19/629

The product

Figure 1: show formulated anti – arthritic cream

Animals

Adult Wistar rats weighing from 120-150 g were used for the study. The Wistar rats were purchased from the animal house facility of Nigeria Natural Medicine Development Agency (NNMDA), Lagos, Nigeria. The study was carried out in NNMDA biomedical laboratory. The Wistar rats were housed and maintained under a controlled room temperature (22 ± 2°C) with a constant humidity (55 ± 5%), ventilation and light (12 h light/12 h dark) cycles and they were fed everyday with rat chow (standard diet) produced by Lodoke feed mills and had access to water *ad libitum*. They were acclimatized to standard laboratory conditions in plastic cages with stainless steel top for seven days prior to the experiment. Toxicity assays were conducted in accordance with the standard guidelines of the Organization for Economic Cooperation and Development (OECD, 2001) for use of animals in scientific research. That is, animal experiments were conducted in compliance with the ARRIVE guidelines for the care and use of laboratory animals in scientific research.

Experimental design for acute toxicity

In acute toxicity study, eighteen (18) Wistar rats weighing from 120 g–150 g were randomly selected for the study and divided into three (3) study groups (n=6 rats/group). The right hindlimb fur of rats in each group were shaved with a clean razor blade under light anaesthesia for topical administration of anti–arthritic cream The experiment protocol is thus; Group 1: control group received 0.2ml saline water topically on the

shaved hind limbs with soft rushes, Group II: received 1g of the anti–arthritic cream topically on the shaved portion of the hind limbs with soft brushes while, Group III: received 1g of the cream base topically on the shaved portion of the hind limbs with soft brushes for 28 days.

Body weight measurements

The weight of the control and Wistar rats in each group were recorded using Mettler PE 1600 analytical balance(+0.01 g, Switzerland) on the first day of the study prior to the topical administration of anti-arthritic cream, and at the end of the experiment the weight of Wistar rats in each group were also determined.

Haematological studies

At the end of the experimental period, euthanasia was administered using Isoflurane ($C_3H_2ClF_5O$, Forane) inhalant followed by gentle cervical dislocation. Euthanized rats were placed in a sealed glass chamber where high levels of anesthetic gas (3 %) were introduced, which depresses the central nervous system resulting in peaceful death. Blood samples were collected once unconsciousness has been achieved. About 3 mL blood samples were collected from each rat in ethylenediamine tetraacetic acid (EDTA) anticoagulant tubes from the retro-orbital vein by a glass capillary tube puncture. Hematological examinations were carried out using Automated Hematology Analyzer (XT-1800i, Japan).

Estimation of biochemical markers

Liver enzymes such as Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT) were assayed as indicators of liver toxicity. This was done by enzyme marker kits using Clinical Chemistry Auto-analyzer (Cobas 6000 analyzer, Germany). The manufacturer's instruction was followed in the course of each biochemical analysis.

Histopathological examinations

Histopathological examinations were done for heart, liver, kidney and lung tissues of the Wistar rats. All rats were sacrificed in a humane manner using Isoflurane anesthesia at the end of the 28th day, the excised tissues were fixed in 10 % buffered neutral formalin solution, and subsequently embedded in paraffin wax. The sections (5 µm thick) were cut using Rotary Microtome 4060E (Germany), and mounted on glass slides and stained with Hematoxylin and Eosin for histopathological investigation.Histopathological changes in the tissues were examined macroscopically for its gross pathological changes upon topical administration of the test sample. All fields of the tissue morphology were examined under light microscope (100x magnification) (Wagtech Thatcham, Berkshire, RG19 4QD, United Kingdom), and the photomicrographs were captured using a digital Camera manually mounted on it (Sony Cyber-shot DSC-W180 10.1 M P with 3x optical Zoom, New Jersey, USA) for further reference.

Statistical analysis

The data were analyzed using Graph Pad Prism version 6 Software (Graph Pad Software, San Diego, CA, USA). Analysis of one way ANOVA followed by Dunnett's comparison test was applied to compare each group with the control. The data values were expressed as mean ± standard deviation (SD). A p-value < 0.05 was considered as statistically significant.

III. Results

Figure 1: show that there was a decrease in the weight of the liver upon topical administration of anti-arthritic cream when compared with the Control Group *($p > 0.05$)* and increase in the weight of the liver upon topical administration of cream base when compared with the Control Group. The increase in the weight of the liver in the Control Group is slight higher than the increase in the weight of the liver in cream base when compared with each other. Topical administration of anti-arthritic cream decreased the weight of the lungs when compared with the Control Group and upon topical administration of the cream base there was an increase in the weight of the lungs compared with the Control Group. Meanwhile, increase in the weight of the lungs in the Control Group is slight higher that the weight of the lungs in the cream base when compared with each other. On the other hand, topical administration of anti-arthritic cream increased weight of the kidney when compared with the Control Group. The increase in the weight of the kidney in the anti-arthritic was similar to the increase in the weight of the kidney in the Control Group when compared with each other. More so, increase in the weight of the kidney in cream base was similar to the increase in the weight of the kidney in the anti-arthritic cream and Control Group when compared with each other. Also, topical administration of anti-arthritic cream increased the weight of pancreas and topical administration of the cream base increased the weight of the pancreas when compared compared with the Control Group. The increase in the weight of the pancreas in the arthritic cream and cream base was similar to increase in the weight of the pancreas in the Control Group when

compared with each other. Heart weight increased upon topical administration of anti-artritic cream likewise increased upon topical administration of cream base when compared with the Control Group. However, the increase in the weight of the heart is in the order of cream base followed by the anti-arthritic and followed by the Control Group

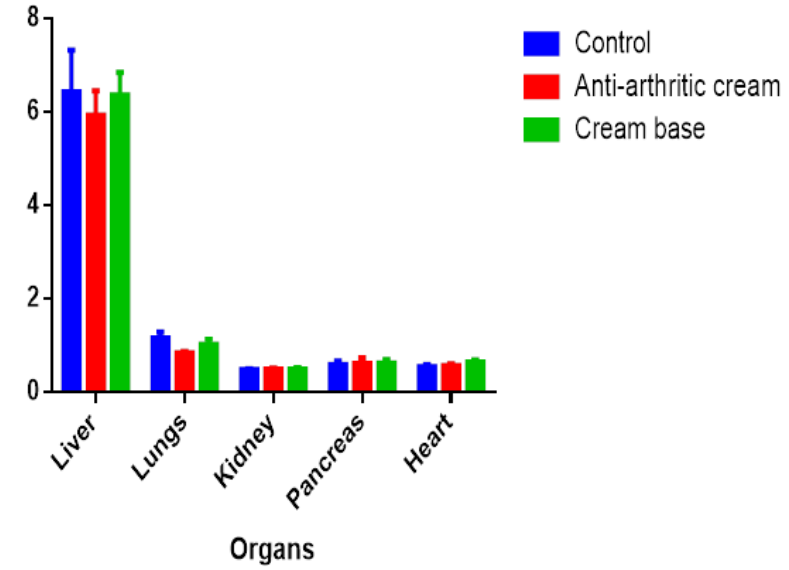

Figure 1: effect of topical application of anti - arthritic cream formulation and cream base on the organs weight in Wistar rats. The results are expressed as a mean standard error n= 6 rats per group. *$p < 0.05$ compared to the control group

Figure 2: show that topical administration of anti-arthritic cream slightly decrease in the serum level of amino asphatate transferase (AST) when compared with the Control Group, but topical administration of cream base increase serum level of (AST) when compared with the Control Group.

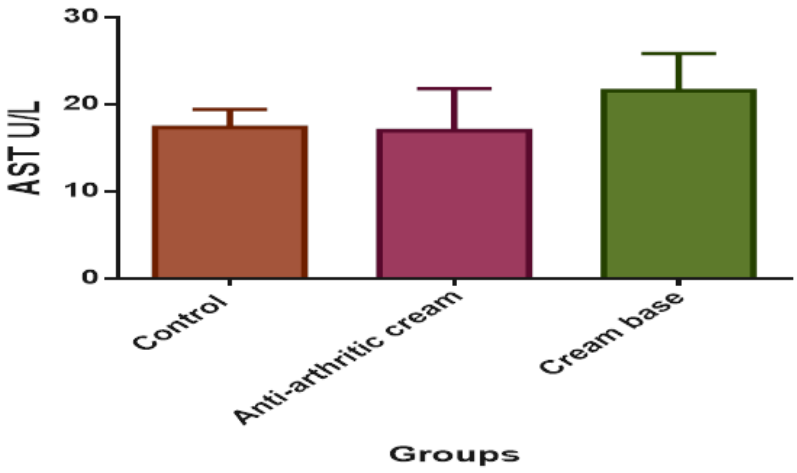

Figure 2: effect of topical application of anti - arthritic cream formulation and cream base on the Alanine asphatate transferase in Wistar rats. The results are expressed as a mean standard error n= 6 rats per group. *$p < 0.05$ compared to the control group

Figure 3: show that topical administration of anti-arthritic cream decrease the level of serum alanine amino trasnsferase (ALT) when compared with the Control Group. But topical administration of cream base increase level of serum (ALT) when compared with the Control Group.The increase in the serum ALT upon topical administration of cream base was similar to the increase in the level of serum ALT in the Control Group.However, there was no significant difference when compared with each other

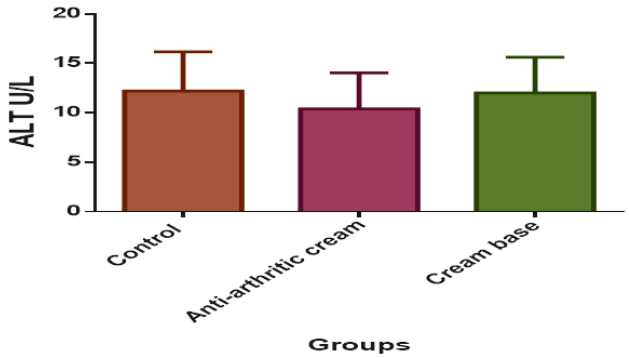

Figure 3: effect of topical application of anti - arthritic cream formulation and cream base on the Alanine amino transferase in albino rats. The results are expressed as a mean standard error n= 6 rats per group. *$p < 0.05$ compared to the control group

Figure 4**:** show that topical administration of anti-arthritic cream increase urea level compared with the Control Group. However, the increase in the urea level of anti- arthritic cream was slight compared to the increase in urea level in the control when compared with each other Meanwhile, topical administration of cream base decrease urea level when compared with the Control Group.

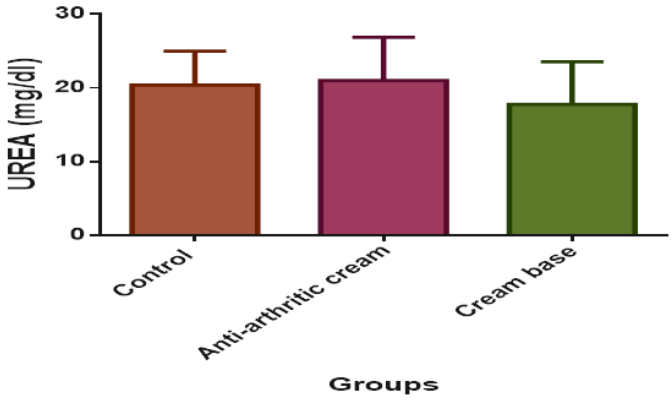

Figure 4: effect of topical application of anti - arthritic cream formulation and cream base on the Urea levels in albino rats. The results are expressed as a mean standard error n= 6 rats per group.*$p < 0.05$ compared to the control group

Figure 5: show that topical administration of anti-arthritic cream increase creatinine level compared with the Control Group. Meanwhile, topical administration of cream base decrease creatinine level when compared with the Control Group. But there was significant (p <0.05) change in topical administration of anti arthritic cream and topical administration of the cream base when compared with each other.

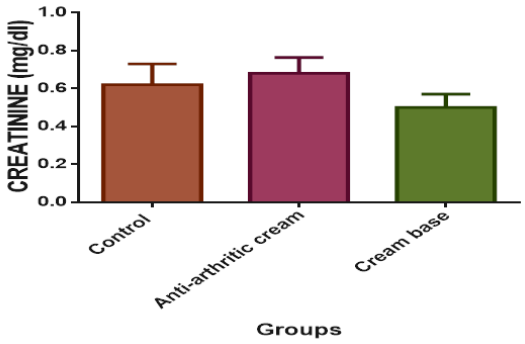

Figure 5: effect of topical application of anti - arthritic cream formulation and cream base on the creatinine levels in albino rats. The results are expressed as a mean standard error n= 6 rats per group.*$p < 0.05$ compared to the control group

Figure 6: show that topical administration of anti-arthritic cream increase low-density lipoprotein (LDL) level compared with the Control Group. Moreso, topical administration of cream base increase LDL level when compared with the Control Group. The increase is in descending order of cream base followed by anti arthritic cream and followed by the Control.

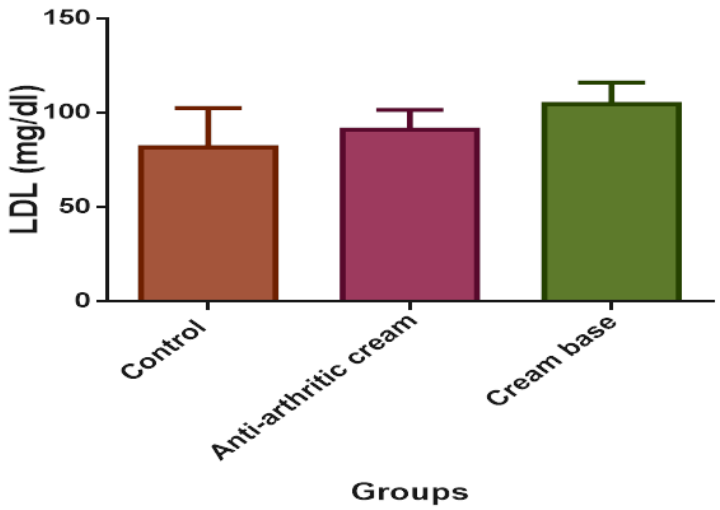

Figure 6: effect of topical application of anti - arthritic cream formulation and cream base on the Low density lipoprotein (LDL) levels in albino rats. The results are expressed as a mean standard error n= 6 rats per group. *$p < 0.05$ compared to the control group

Figure 7: show that topical administration of anti-arthritic cream slight decrease in high-density lipoprotein (HDL) level compared with the Control Group. but topical administration of cream base increase HDL level when compared with the Control Group ($p < 0.05$).

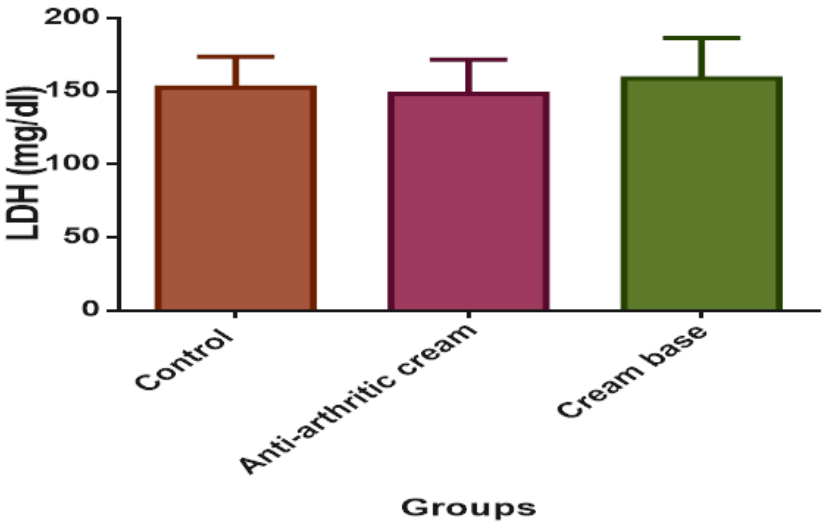

Figure 7: effect of topical application of anti - arthritic cream formulation and cream base on the high density lipoprotein (HDL) levels in albino rats. The results are expressed as a mean standard error n= 6 rats per group.*$p < 0.05$ compared to the control group

Figure 8: show that topical administration of anti-arthritic cream and topical administration of cream decreases white blood level compared when compared with the Control Group ($p < 0.05$ in each case). The decrease in the white blood cell level is in the ascending order of cream base, followed by the anti-arthritic cream, followed by the Control wen compared wit each other.

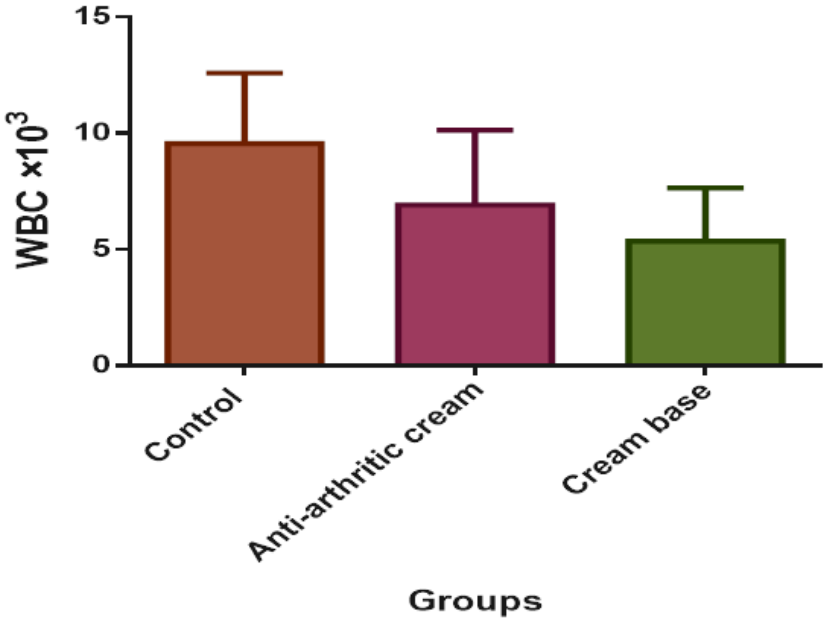

Figure 8: effect of topical application of anti - arthritic cream formulation and cream base on the white blood cell (WBC) levels in albino rats. The results are expressed as a mean standard error n= 6 rats per group. *$p < 0.05$ compared to the control group

Figure 9: show that topical administration of anti-arthritic cream decrease red blood level compared when compared with the Control Group ($p < 0.05$). while topical administration of cream base increase red blood level when compared with the Control Group ($P < 0.05$).

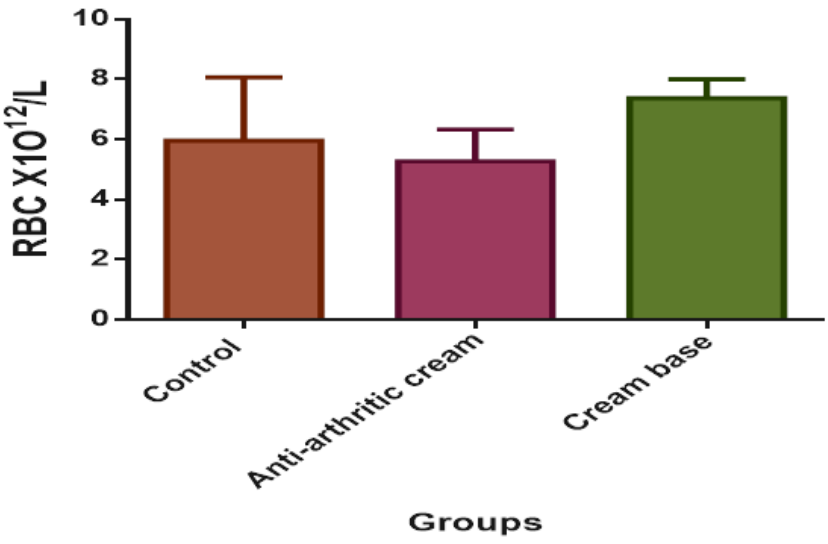

Figure 9: effect of topical application of anti - arthritic cream formulation and cream base on the red blood cell (RBC) levels in albino rats. The results are expressed as a mean standard error n= 6 rats per group. *$p < 0.05$ compared to the control group

Figure 10: show that topical administration of anti-arthritic cream increase blood haemoglobin concentration when compared with the Control Group and topical administration of cream base decrease blood haemoglobin concentration when compared with the Control Group

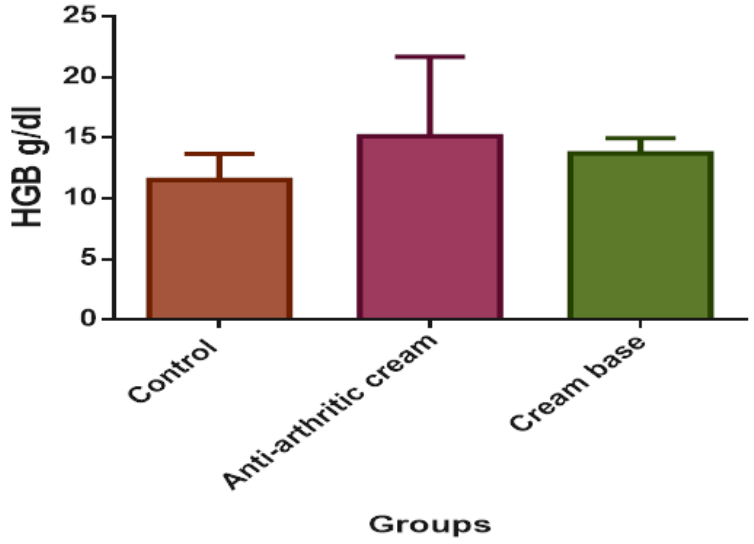

Figure 10: effect of topical application of anti - arthritic cream formulation on the haemoglobin (HBG) levels in albino rats. The results are expressed as a mean standard error n= 6 rats per group. *p<0.05 compared to the Control Group

Figure 11: show that topical administration of anti-arthritic cream decrease the haematocrit level when compared with the Control Group ($p < 0.05$),while topical administration of cream base increase haematocrit level when compared with the Control Group.

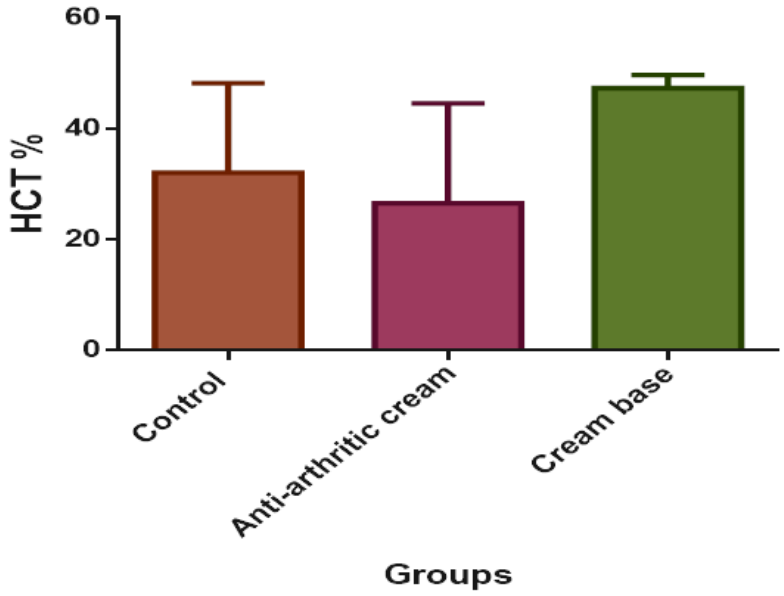

Figure 11: effect of topical application of anti - arthritic cream formulation on the white haematocrit (HCT) levels in albino rats. The results are expressed as a mean standard error n= 6 rats per group. *p<0.05 compared to the control group

Figure 12: show that topical administration of anti-arthritic cream increase platelets level when compared with the Control Group. Topical administration of cream base increase platelets level when compared with the Control Group. There was no significant increase in the platelets level when compared with eachother

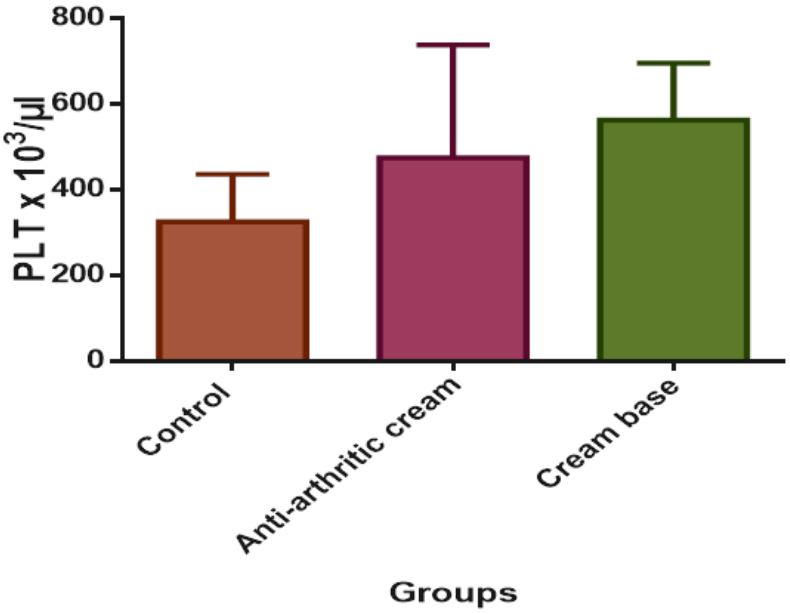

Figure 12: effect of topical application of anti-arthritic cream formulation on the platelets formation (PLT) levels in albino rats. The results are expressed as a mean standard error n= 6 rats per group. *p<0.05 compared to the control group

Figure 13: show that topical administration of anti-arthritic cream increase packed cell volume when compared with the Control Group.,while topical administration of cream base increase packed cell volume when compared with the Control Group, the increase in the packed cell volume in anti-arthritic cream and the cream base are not significant when compared with each other.

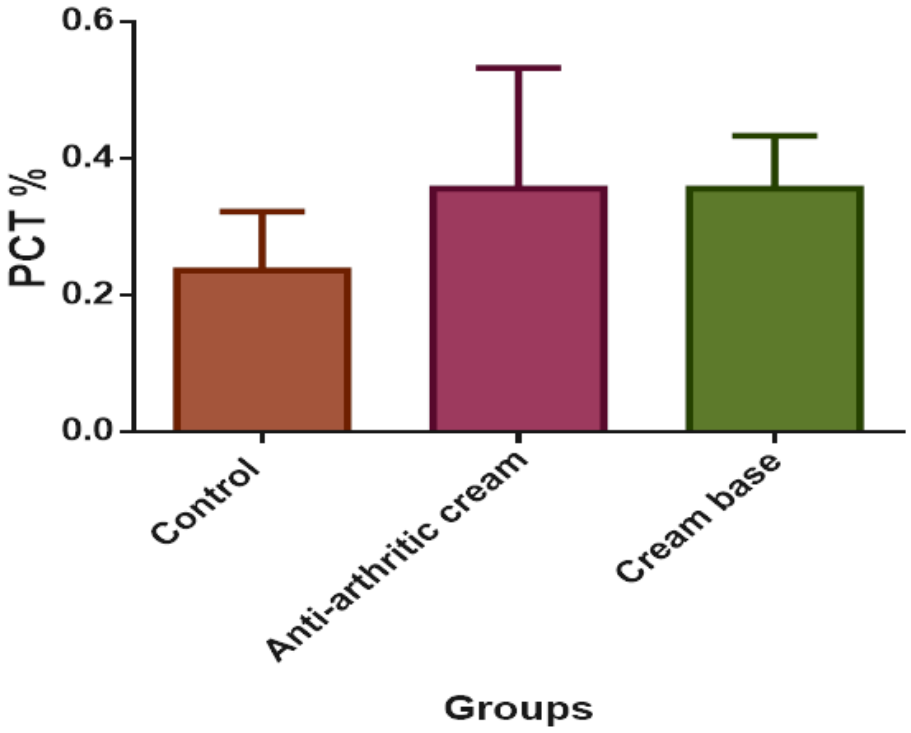

Figure 13: effect of topical application of anti-arthritic cream formulation on the packed cell volume (PCT) levels in albino rats. The results are expressed as a mean standard error n= 6 rats per group. *p<0.05 compared to the control group

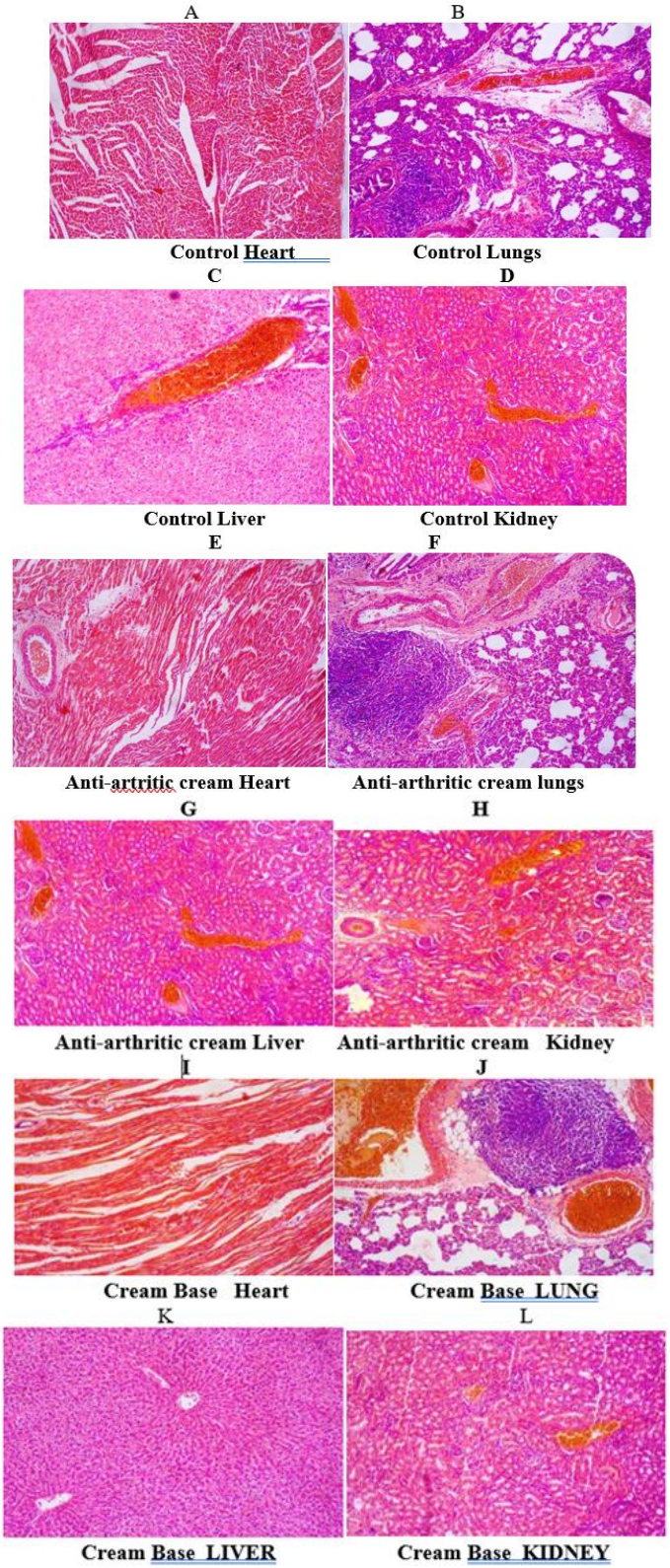

Figure 14 (A-L): show histological photomicrograps of the heart, lungs, liver and kidney after topical administration of anti-arthritic cream and topical application of cream base in Wistasr rats (x100). Control (A-D) showing normal architectures of the heart, lungs, liver and kidney. Topical administration of anti -arthritic cream(E-H) showing heart histologic sections of myocardial inflammation; Lungs histologic section showing vascular congestion; liver histologic section showing vascular congestion and Kidney histologic section showing vascular congestion. Topical administration of cream base (I-L) showing heart histologic section showing myocardial inflammation; lungs histologic sections showing vascular congestion; Liver histologic section showing No abnormalities are seen and kidney histologic section showing vacular congestion

IV. Discussion

The study investigated acute toxicity studies of topical administration of anti-arthritic cream and topical administration of cream base in Wistar rats. Our findings show decrease in the weight of the liver and lungs when treated with topical administration of anti-arthritic cream. Physiologically, decrease in the weight of the liver and lungs are associated with reduction in inflammation which may be beneficial in conditions such as hepatitis and pneumonia, therefore, decrease in both liver weight and lungs weight, may be attributed to the ability of anti-arthrtitic cream in reducing or inhibiting some inflammatory agents such as interleukin-1 beta (1L-1ß), tumor necrosis factor -alpha (TFN-α), and interleukin -6 (IL-6), thromboxanes e.g (TXA2), postagladins (PGE2), and nuclear factor kappa B activator therefore topical administration of anti-arthritic cream does not have deleterious effects on both liver and lung functions which indicates liver and lungs protections. Liver and kidney are primary organs affected by metabolic reaction [9,2] and are useful in predicting toxicity effects of phytotherapy products[10]. Liver is the main target for toxic compounds because of its prior exposure to the foreign substances absorbed in the intestine before reaching into the blood circulation[12,13]. although toxins may harm the liver, it detoxifies toxin[14], thus in the exprimental animals, liver function test would allow to understand the toxic effect, which can be extrapolated for safety if used in humans. Alanine amino transferase (ALT) and aspartate amino transferase (AST), are enzymes that are commonly used to assess liver health conditions. ALT is relatively specific to liver and a useful marker for liver damage, but AST is less specific to the liver and can be elevated in various conditions affecting different tissues. Significant decrease in the creatinine by the anti-athritic cream and cream base indicates in one part that, topical administration of anti-arthritic cream may not affect kidney functions while on the second part, cream base compliment the activities of anti-arthritic cream in maintaining kindy function. The chemical composition of anti-arthritic cream possesses, capsaicin, dihydrocapsaicin, limonene, phenolic acid, flavonoids, saponin, glycyrrhizin, glycyrrhetic acid, mineral oil, petrolatum, dimethicone, panthenol, kaempferol, quercetin, luteolin and menthol. The activities of anti-arthritic cream is attributed to its antioxidant properties as show in its chemical composition.Topical administration of Anti-arthritic cream and topical administration of cream base significantly reduced white blood cell and red blood cell which implies that upon the use of arthritic cream, it cant not cause blood anaemia. On the contrary, the significant reduction in the white blood cell indicate that the anti arthritic cream may reduce body immune system upon a long time usage. While the photocromatograph of the tissue show that anti arthritic cream does not cause tissue damage. Finally finding in this study revealed that topical administration of anti-arthritic cream does not cause any deleterious effects on the bodily vital organs, since there was no tissue necrosis occurred. Also, caution must be undertaken on the long time use of anti-arthritic cream due to it inability to improve red blood cell and white blood cell counts

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